Population genetic consequences of mating systems and dispersal

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Declaration of any previous submission of the work

The material presented here for examination for the award of a higher degree by research has not been incorporated into a submission for another degree.

Candidate's signature ……..

Declaration of authorship

I am the author of this thesis, and the work described therein was carried out by myself personally, with the exception of Chapters 2-5 (data chapters) where my contribution was between 75-90%. In these chapters 25-10% of the work was carried out by co-authors, see the Statement of Authorship before each chapter for details.

Candidate's signature……..

Contents

A thesis commentary is presented before and after Chapters 2-5.

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The Silverfish Rap
Anthologised in 'Don't Step On That Earwig' Hutchinson 1992
By Rowena Sommerville

Well I
opened up the cupboard
just to get a can of corn
and sitting in the corner
all forsaken and forlorn
was a tiny little silverfish
a-shiverin on the shelf
he was mumbling and a–grumblin
and a–talkin to himself.

He said -
a thousand thousand years
and then a thousand thousand more
and a thousand thousand creatures
looking different from before
and some are looking uglier
and some are looking swish,
but me and mine we stay the same
we're stuck as silverfish.

While some dinosaurs are flying
and some are climbing trees
and some are growing fingers
and some grow wrinkly knees,
but God decided on this evolutionary
niche
and said, 'By George, I've got it!
You're a perfect silverfish!'

I don't
like to keep complaining
but each day it's getting harder,
and my world view is restricted
to the inside of the larder,
I'm not asking for a miracle,
I don't want revolution
but I'd like a small development,
I'd like some evolution.

And I'd
like to climb a mountain
and to see what 'sunrise' means,
and I'd like to read some books
not just the labels on the beans,
and I'd like to swim the ocean
and I'd like to play the drums
and I'd like a change from sitting
in this cupboard, eating crumbs.

Well I
closed the cupboard door
and then I made a cup of tea
and I thought about this creature
and its static history,
and if I think my life is dull,
and not just what I'd wish
I'll still be very grateful
that I'm not a silverfish!

Summary

Understanding the drivers of genetic differentiation in natural populations is one of the fundamental goals in evolutionary biology. The maintenance of genetic diversity is essential for the persistence of species and it may require dispersal between populations. In this dissertation I focus on dispersal, mating systems, and their population genetic consequences in evolutionary and conservation biology. Using shorebirds (plovers, sandpipers and allies) that exhibit an unusual diversity in mating system, I explore the relationships between dispersal, mating systems, and genetic diversity from the chromosome, to a macro-evolutionary scale. First, my dissertation shows that contrary to the expectation, intense sexual selection can reduce rather than increase population differentiation, by increasing the dispersal of individuals between populations to increase their mate access. Second, I show that with intense sexual selection on females, the genetic diversity of the Z chromosome is increased and the opportunity for genetic drift is reduced. The latter finding contrasts with previous work that has been restricted to polygynous or monogamous mating systems, and demonstrates that the genetic consequences of mating systems may depend on both the direction and the intensity of sexual selection acting on males relative to females. Third, using demographic modelling, I found signals of population bottlenecks in a threatened shorebird, the snowy plover (*Charadrius nivosus*), and used complementary genetic markers to highlight subspecies for conservation prioritization. Fourth, by combining field and genetic data, I found signals of population expansion in the Madagascar jacana (*Actophilornis albinucha*), suggesting this vulnerable species has maintained genetic diversity, despite on-going habitat destruction. Taken together, my dissertation demonstrates that the consequences of mating systems are complex and may impact across several evolutionary scales. Future investigations should combine theoretical and empirical approaches using field and molecular data, to disentangle the mechanisms behind the relationship between mating systems, dispersal, and diversification.

1

Introduction

Sexual selection and speciation

Our fascination with the origins of biodiversity drives the field of evolutionary biology. Pre and post Darwin and Wallace, biologists have endeavoured to answer fundamental questions of how, why, and when species evolved. Although the diversification of species through natural selection is well established, the role of sexual selection in speciation remains highly debated due to conflicting theoretical and empirical evidence (Coyne and Orr 1998, Price 1998, Panhuis *et al.* 2001, Ritchie 2007, Maan and Seehausen 2011, Kraaijeveld *et al.* 2011, Servedio 2016, Servedio and Boughman 2017, Janicke *et al.* 2018).

Sexual selection is intrinsically linked to mating systems and sex roles (Emlen and Oring 1977, Shuster and Wade 2003, Shuster 2009, Janicke *et al.* 2016). Polygamous species experience greater variance in mating success than monogamous species (Emlen and Oring 1977) and this variance drives males (in polygynous species) or females (in classically polyandrous species) to compete with others of the same sex for access to the opposite sex (Darwin 1871). Access to the opposite sex can be improved by becoming more attractive, for example, evolving exaggerated traits through "Fisherian runaway selection" (Fisher 1930). When mate choice and sexual traits genetically covary (Fowler-Finn and Rodríguez 2016), sexual selection promotes speciation though diversifying selection and prezygotic reproductive isolation (Lande 1981, Kirkpatrick 1982, West-Eberhard 1983, Panhuis *et al.* 2001, Ritchie 2007). Through this process sexual selection can be termed an "engine of speciation".

Although a number of theoretical (e.g. Higashi *et al.*, 1999) and empirical studies (e.g. Mitra *et al.* 1996, Krüger 2008, Kraaijeveld *et al.* 2011, Wagner *et al.* 2012, Seddon *et al.* 2013, Ellis and Oakley 2016, Janicke *et al.* 2018) claimed to have found support for sexual selection as a facilitator of speciation, there is an increasing body of evidence which questions how dominant sexual selection is in driving the emergence of new species (e.g. Gage *et al.* 2002, Morrow *et al.* 2003, Kirkpatrick and Nuismer 2004, Servedio and Kopp 2012, Huang and Rabosky 2014, Servedio and Bürger 2014). Alternative hypotheses have been suggested, for example, Morrow *et al.* (2003) argue that sexual selection may increase both speciation and extinction rates, leaving no evidence of the diversity it has produced. Whereas, Gage *et al.* (2002) suggest that mating systems may arise after speciation has occurred rather than driving speciation patterns. Alternatively, others explain that strong sexual selection alone is not powerful enough to promote speciation

(Van Doorn *et al.*, 2004, Servedio, 2011, Servedio & Kopp, 2012, Servedio and Burger, 2014) and in some cases it is suggested that sexual selection can in fact inhibit the forces of divergence (Servedio 2016).

Mating systems and dispersal

One way in which sexual selection pressure can reduce or inhibit population divergence may be through increased mate searching of polygamous compared to monogamous species. Dispersal is a plastic trait (Arendt 2015) which allows individuals to take advantage of spatially and temporally fluctuating resources, to increase their fitness (Clobert *et al.* 2012). In sexual species, mating partners are vital resources to exchange genetic material, therefore, to increase the opportunity for mating success, increased movement may be required. Understanding the relationship between dispersal and mating systems is central to interpreting how sexual selection can potentially limit speciation.

In his seminal work, Greenwood (1980) compared bird and mammal dispersal strategies in terms of mating systems. In birds, which he defined as majority monogamous species, Greenwood (1980) hypothesised that males benefit from philopatry through the acquisition of high-quality territory which attracts dispersing females (also known as resource defence). Whereas, in polygynous and polygynandrous mammals, which are under greater sexual selection pressure, males disperse to access multiple females though male-male competition, also known as the "mate defence" strategy. Greenwood's hypotheses have received mixed support (reviewed by Clarke *et al.* 1997, Lawson Handley and Perrin 2007, Dobson 2013, Mabry *et al.* 2013, Trochet *et al.* 2016) and additional explanations for the relationship between mating systems and sex-biased dispersal include: competitive avoidance by kin (Dobson 1982, Pusey 1987, Perrin and Mazalov 2000, Waser *et al.* 2013, Brom *et al.* 2016) and inbreeding avoidance by the limiting sex (Pusey 1987, Perrin and Mazalov 2000, Trochet *et al.* 2016).

Increased local competition for the limiting sex may also promote their dispersal (Dobson 1982, Végvári *et al.* 2018) due to the combined result of high pressure to breed with multiple partners and skewed sex ratios (Emlen and Oring 1977, Janicke and Morrow 2018). Supporting this, evidence of high breeding dispersal in polygamous species has been reported for a number of birds and mammals (e.g. Székely and Lessells 1993, Stenzel *et al.* 1994, Blundell *et al.* 2002, Gauffre *et al.* 2009, Kempenaers and Valcu 2017). However, empirical evidence for sexual selection driving different breeding dispersal patterns is limited (Végvári *et al.* 2018), despite this potentially having the capability to directly influence speciation (Maan and Seehausen 2011, Clobert *et al.* 2012).

Genetic measures of mating systems and dispersal

Directly measuring dispersal across multiple populations is challenging for a number of reasons, including requiring expensive tracking devices which can limit the samples size of the study, or long term investment to recapture marked individuals (reviewed by Broquet and Petit 2009, Griesser *et al.* 2014, Cayuela *et al.* 2018,). Alternatively, genetic tools can be used to estimate dispersal, directly, by using population assignment (reviewed by Manel *et al.* 2005), or indirectly using gene-flow as an approximate

measure if dispersal is followed by successful reproduction (i.e. effective dispersal) (Slatkin 1987). In these ways, genetic methods can significantly increase the power of dispersal estimates as well as reduce the time and financial investment required for tracking animal and plant movements (Broquet and Petit 2009). Furthermore, reductions in the cost of sequencing and the development of molecular methods such as restriction site associated DNA sequencing (RADseq, Andrews and Luikart 2014, Baird *et al.* 2008, Miller *et al.* 2007) have increased our ability to detect subtle signatures of dispersal in non-model organisms (Cayuela *et al.* 2018).

Similarly, genetic markers have been used to detect mating systems without direct observation of mating events since the development of genetic fingerprinting (Jeffreys *et al.* 1985), which facilitated individual identification and parentage analysis in wild species (Burke and Bruford 1987, Burke *et al.* 1989). Molecular tools, in combination with field studies have demonstrated the huge diversity of mating systems found in nature (Hugues 1998), including the ubiquity of female promiscuity in socially monogamous bird species (Petrie and Kempenaers 1998), polyandry in sex-role reversed species (Emlen *et al.* 1998, Jones *et al.* 2001), extreme polygyny (Fabiani *et al.* 2004) and inter- and intra-population mating system variation (Coltman *et al.* 1999, Hammond *et al.* 2001).

Genetic consequences of mating systems

Population differentiation

Dispersal can directly influence speciation rates through either limiting (low dispersal) or increasing (high dispersal) the exchange of genetic material between populations (Belliure *et al.* 2000, Claramunt *et al.* 2012, Riginos *et al.* 2014, Weeks and Claramunt 2014, Jønsson *et al.* 2016, Medina *et al.* 2018). A reduction in gene-flow can lead to increased population differentiation, which is a precursor to speciation (Mallet 1995, Harvey *et al.* 2017).

Mate searching and other breeding tactics can be reflected in gene-flow estimates and genetic structure (Chesser 1991, Clark *et al.* 2008, Parreira and Chikhi 2015, Jahner *et al.* 2016, Morinha *et al.* 2017, Shaw *et al.* 2018). For example, recent evidence from red-billed choughs (*Pyrrhocorax pyrrhocorax*), which are highly mate faithful (Banda and Blanco 2014), has revealed highly socially structured genetic differentiation patterns (Morinha *et al.* 2017). The use of genetic tools to detect sex-biased dispersal (Goudet *et al.* 2002, Prugnolle and Meeûs 2002, Cooper *et al.* 2010, Banks and Peakall 2012) has revealed genetic signatures of mating strategies across taxa (e.g. Blyton *et al.* 2015, Chapple and Keogh 2005, Höner *et al.* 2007, Portnoy *et al.* 2015, Taylor *et al.* 2003). Simulations of genetic data from sex specific regions have also improved our understanding of mating systems and dispersal strategies (e.g. Shaw *et al.* 2018). Genomic tools significantly increase the power to detect subtle patterns that may otherwise go unnoticed. For example, fine scale genetic structure of greater sage-grouse (*Centrocercus urophasianus*) leks was only evident with over 4000 loci (Jahner *et al.* 2016). Similarly, in Emperor penguins (*Aptenodytes forsteri*) subtle genetic structuring was revealed after substantially increasing the number of markers, sampling sites, and individuals (Younger *et al.* 2017). Furthermore, techniques such as RADseq can provide a large number of sex specific markers for the estimation of sex-biased dispersal

patterns (e.g. Aguillon *et al.* 2017, Dai *et al.* 2013, Lavretsky *et al.* 2015), which is especially challenging in highly vagile taxa such as birds due to high overall gene-flow. Molecular methods, therefore, have not only transformed our ability to predict mating systems, but they also provide inferences of the consequences of breeding behaviours at micro- and macro-evolutionary scales (e.g. Storz *et al.* 2001, Corl and Ellegren 2012, Shaw *et al.* 2018).

Our understanding of the behavioural drivers of dispersal is not only important for disentangling demographic and genetic patterns but also has important implications in conservation management (reviewed by Driscoll *et al.* 2014). For example, traits that predispose a species to high or low dispersal should be considered for effective translocations (Hall *et al.* 2010) or for reintroduction programmes, especially when hybridisation threatens a species' survival (Fredrickson and Hedrick 2006). However, identifying overarching behavioural traits that result in consistent genetic patterns across species, remains one of the challenges of behavioural evolutionary research, especially in species with high gene-flow, for two main reasons. Firstly, it requires comparable genetic data to be available for multiple species which differ in behaviour, but share traits such as dispersal ability and phylogenetic history. Secondly, detecting the subtle genetic structure might only be possible with many hundreds or thousands of markers in taxa characterised by high gene-flow (Friesen *et al.* 2007, Jahner *et al.* 2016, Medina *et al.* 2018). Although these datasets are becoming more frequently available, they are still limited by sample size for comparative investigations.

Intra-genomic variation

Mating systems not only have the power to shape gene-flow patterns via dispersal but also to influence the genetic diversity of sex specific regions of the genome itself (e.g. Charlesworth 2001, Corl and Ellegren 2012, Irwin 2018, Verkuil *et al.* 2014, Wright *et al.* 2015). The two most common sex determining systems are XY (mammals and drosophila) and ZW (birds and Lepidoptera), where males are the heterogametic sex in XY systems and females in ZW systems. Mitochondrial DNA (mtDNA) is largely maternally inherited in both systems (Barr *et al.* 2005). Effective population size is directly related to genetic diversity (Frankham 1996b, Soulé 1976), therefore, unequal sex ratios and sex specific variance in reproductive success can have a differential effect on the autosomes and sex specific regions (Charlesworth 2001, Wilson Sayres 2018). For example, in polygynous ZW species, high variance in mating success leads to a smaller relative effective population size on the Z chromosome compared to the autosomes, than is expected under neutrality (Corl and Ellegren 2012), but higher diversity in mtDNA compared to the autosomes (Verkuil *et al.* 2014). Polygyny has also been associated with driving "Fast-Z" evolution (reviewed by Meisel and Connallon 2013). This is the elevated rate of divergence of the Z chromosome compared to the autosome is due to a reduction in effective population size, leading to weakened purifying selection and faster genetic drift (Wright *et al.* 2015). Sexual selection has also been directly implicated in causing a high turnover of male biased, but not female biased, gene expression in polygynous birds (Harrison *et al.* 2015). It is evident, however, that since its conception, our understanding of the population genetic consequences of mating systems has been dominated by studies focusing on polygyny rather than polyandry.

"This [sexual selection] depends not on the struggle for existence, but on the struggle between males for possession of females." Darwin (1871)

Polyandry

Broadly defined as female promiscuity, polyandry is widespread in nature (Taylor *et al.* 2014) and has received a recent surge in attention (Andersson 2005, Rosvall 2011, Boulton and Shuker 2013, Kvarnemo and Simmons 2013, Parker and Birkhead 2013, Pizzari and Wedell 2013). Consequences of this mating system have been proposed at every evolutionary scale. Polyandry has been associated with increased speciation rates in insects via to antagonistic coevolution of sex specific traits (Arnqvist *et al.* 2000), however, Gage et al (2002) found no such relationship in butterflies or mammals and suggested polyandry system evolves independently of speciation processes. Across taxa, multiple paternity is also associated with high genetic diversity overall (Petrie *et al.* 1998, Balloux and Lehmann 2003, Oldroyd and Fewell 2007, Slatyer *et al.* 2012, Taylor *et al.* 2014), as well as in specific genomic regions, for example, the major histocompatibility complex (Winternitz *et al.* 2013). Elevated genetic diversity (Frankham 2005), is one of at least three ways polyandry is proposed to reduce extinction risk, the second is by reducing mutation load and speeding up adaptation through increased sexual selection, although the evidence for this is mixed (reviewed by Holman and Kokko 2013), and thirdly by reducing the frequency of sex-linked meiotic drive which can lead to single sex broods (Price *et al.* 2010). At a genomic level, polyandry has the potential to cause sex-specific differences in gene expression, however, empirical evidence of this is limited (reviewed by Mank *et al.* 2013).

Classical polyandry, which is the reversal of conventional sex roles (Trivers 1972), whereby females compete more intensely than males for mating opportunities and males perform the majority of parental care, is comparatively far less common than social monogamy or polygyny (Kvarnemo and Simmons 2013). Most examples of classical polyandry are found in fish and birds (Andersson 2005), however, within these groups male only care is still rare, for example, it occurs in just 1% of all avian species (Lack 1968, Cockburn 2006). The reversal of sex roles has intrigued biologists for many years, being described as the most interesting of avian mating systems (Ligon 1999), though it remains not well understood (reviewed by Andersson 1995, 2005, Eens and Pinxten 2000, Owens 2002). Classical polyandrous behaviour can involve sequential (e.g. snowy plover, *Charadrius nivosus*, Warriner *et al.* 1986; Kentish plover *Charadrius alexandrinus,* Lessells 1984) or simultaneous (e.g. African jacana, *Actophilornis africanus,* Vernon 1973) breeding events per season. Multiple hypotheses have been described to explain the evolution of classical polyandry (Jenni, 1974, Emlen and Oring 1977, Graul 1977, Owens 2002, Andersson 2005, Liker *et al.* 2013), including the stepping stone model (First, male care increases, second, female ability to produce multiple clutches increases beyond the number of offspring than one male can care for, and finally, third, females compete to lay multiple clutches for different males (Andersson 2005). However, empirical support for the stepwise model to sex role reversal is lacking (Clutton-Brock 1991, Bennett and Owens 2002). Currently, the most well supported hypothesis for the evolution of classical polyandry is based on male biased adult sex-ratios (Emlen and Oring 1977, Andersson 1994, 2005, Liker *et al.* 2013, Goymann *et al.* 2016), which lead to high reproductive competition among females for mate access. As females are the

limiting sex, there are fewer opportunities for males to re-mate, therefore, males evolve parental care, while females are emancipated from their care duties and able to breed with additional males (Liker *et al.* 2013).

Genetic consequences of classical polyandry in ZW systems are likely to arise from the associated male biased adult sex ratio and high female reproductive skew (Emlen and Oring 1977, Andersson 1994, 2005, Kvarnemo and Simmons 2013). As a result, the effective population size of the Z chromosome is higher compared to neutral expectations (equal sex ratio and equal mating success, Charlesworth 2001). As opposed to polygynous ZW scenarios, under polyandry the larger effective population size strengthens the effect of purifying selection, therefore, weakening the process of genetic drift between populations (equivalent of polygyny in XY systems, Laporte and Charlesworth 2002, Vicoso and Charlesworth 2009, Wright and Mank 2013). Accordingly, we would expect in similar levels of population divergence on the Z chromosome compared to the autosome in polyandrous species, unless the efficacy of positive selection is increased (Sackton *et al.* 2014). However, our understanding of how male biased sex ratio and female reproductive skew influences genomic diversity and population divergence is severely limited (Corl and Ellegren 2012, Irwin 2018). To my knowledge, the relative Z chromosome to autosome diversity (N_Z/N_A ratio) has only been studied in one classically polyandrous species, the red necked phalarope (*Phalaropus lobatus*, Corl and Ellegren 2012). Although the N_Z/N_A ratio of the red necked phalarope was the highest among polygynous and monogamous species comparisons, the ratio was not greater than expected under neutrality (Corl and Ellegren 2012). This could be explained by a low frequency of polyandry in this species and a female, rather than male, biased operational sex ratio (Whitfield 1990), which together may constrain the effective population size of the Z chromosome. This species is, therefore, not an ideal candidate to study the influence classical polyandry has on genomic diversity. Furthermore, no investigation, to my knowledge, has investigated how this mating system may influence intra- and inter-specific divergence patterns of the autosomes and the Z chromosome.

Genetic diversity, demography and conservation

Evolutionary processes are now commonly included in conservation management discussions (Crandall *et al.* 2000, Willi *et al.* 2006, Thomassen *et al.* 2011, Garrick *et al.* 2015, Pierson *et al.* 2015). Low genetic diversity often corresponds to high extinction risk in wild populations (Spielman *et al.* 2004, Frankham 2005, O'Grady *et al.* 2006, Evans and Sheldon 2008) and reduced adaptive potential to changing environments (Willi *et al.* 2006). However, present day genetic diversity could be the result of a multitude of complex factors that have occurred over large timescales including, inbreeding, genetic drift, population bottlenecks and founder events (Frankham 1996b). Distinguishing "recently rare" from "naturally rare" populations by reconstructing demographic histories can help us prioritize the most vulnerable populations, which have undergone population contractions as a result of human activities (Garrick *et al.* 2015). Reconstructing complex demographic histories is increasingly common in conservation genetic studies (Carnaval *et al.* 2009, Palsbøll *et al.* 2013, Shafer *et al.* 2015, Stoffel *et al.* 2018) thanks to rapid methodological and software developments (e.g. Beaumont, 2010, Csilléry *et al.*, 2010). Although demographic modelling can greatly

improve our ability to characterise genetic divergence and population connectivity, both of which are central to the delineation of conservation units (Moritz 1994, Palsbøll *et al.* 2007), the use of them in prioritizing conservation units remains relatively recent (Lopes and Boessenkool 2010, Stockwell *et al.* 2013).

There are two main types of conservation unit, evolutionary significant units (ESUs) and management units (MUs, Moritz 1994). The definitions of both are debated but in general, ESUs are groups of individuals which are reciprocally monophyletic at mitochondrial DNA (mtDNA) sequences and are significantly divergent at nuclear loci (Moritz 1994, Fraser and Bernatchez 2001). Management units on the other hand are demographically independent and distinct from others in their genetic diversity at a finer scale (e.g. significant allelic frequency divergences at the nuclear level), but which share haplotypes of slower mutating markers (e.g. mtDNA) with other MUs (Moritz 1994, Palsbøll *et al.* 2007). These units, or derivatives of them, can hold strong legislative power to protect populations and allocate resources, including in the USA, Australia, and Canada. Therefore, the accuracy of ESU and MU classifications is of utmost importance in biodiversity conservation (Haig *et al.* 2011). However, the delineation of these units is highly nuanced and difficult to define in practice because of species specific ecology and life history traits. For example, highly dispersive taxa such as birds are unlikely to have as many reciprocally monophyletic groups at mtDNA, simply due to high rates of gene-flow (Crandall *et al.* 2000, Medina *et al.* 2018). In addition, the incorporation of adaptive genetic diversity into defining conservation units (Funk *et al.* 2012) has been met with strong debate (Prince *et al.* 2017, Langin 2018), however, recommendations for scientists, managers and policy makers are beginning to take shape (Barbosa *et al.* 2018, Flanagan *et al.* 2018, Funk *et al.* 2018).

Study systems

To study the genetic consequences of mating systems I will focus on shorebirds (Suborders: Charadrii, Chionidi, Scolopaci and Thinorcori). Shorebirds (approx. 250 species), or waders, are a highly diverse and well-studied group of birds which display a huge variation in mating systems and dispersal behaviour (Burger and Olla 1984). The unrivalled spectrum of breeding behaviour in shorebirds spans from extreme polygyny and female only care (e.g. *Philomachus pugnax*) to biparental care and monogamy (e.g. *Himantopus himantopus*), to polyandry and near complete sex-role reversal (e.g. *Jacana jacana*) (Székely *et al.* 2006). As a result, shorebirds offer ample opportunity for biologists to study the evolution of mating systems. Furthermore, several additional traits of shorebirds predispose them to act as model systems for the study of breeding behaviour. For example, shorebirds exhibit nearly the full spectrum of sexual size dimorphism of all birds (Székely et al. 2007), and even their their egg characteristics are uniquely variable among birds (Stoddard et al. 2017). Most shorebirds breed and forage in open environments, enabling the capture and re-sighting of known individuals. Since the 1980s (Greenwood 1980, Greenwood and Harvey 1982), determining a link between mating systems, dispersal and breeding success has been a topic of great curiosity for ornithologists including shorebird ecologists (e.g. Stenzel *et al.* 1994, Robinson and Oring 1997, Saalfeld and Lanctot 2015). High site and mate fidelity has been described in monogamous arctic shorebirds (Pitelka *et al.* 1974, Saalfeld and Lanctot 2015), whereas, polygamous shorebirds are known to visit several breeding sites during their

reproductive season (e.g. Kempenaers and Valcu 2017) and have a generally panmictic genetic structure (e.g. Küpper *et al.* 2012, Verkuil *et al.* 2014). However, few have examined how mating system driven dispersal patterns could influence micro- and macro-evolutionary patterns such as population divergence and speciation (Küpper *et al.* 2012, Eberhart-Phillips *et al.* 2015, Kempenaers and Valcu 2017).

Shorebirds have high global importance as environmental indicators (Piersma and Lindström 2004) and are key species in coastal and wetland food chains (Hean *et al.* 2017). As reservoirs of zoonotic diseases (e.g. avian influenza, Olsen *et al.* 2006, Gaidet *et al.* 2012, Miller *et al.* 2015) with high dispersal capabilities, understanding the movements of shorebirds is significant to human and wildlife health. However, many shorebird species live in fragile ecosystems and are threatened by a multitude of factors including land use change, pollution and climate change (Galbraith *et al.* 2002, Sutherland *et al.* 2012). Identifying promotor and restrictors to their movements is therefore not only central to improving our understanding of evolutionary processes but it is also crucial to ensure their persistence (Haig *et al.* 1998, 2016, Amezaga *et al.* 2002, Kool *et al.* 2013, Merken *et al.* 2015, Lean *et al.* 2017).

Plovers (Genus: Charadrius)

Within shorebirds, plovers constitute an ideal group of taxa to test hypotheses concerning the population genetic consequences of mating systems and dispersal for the following reasons: i) their evolutionary relationships have recently been described (dos Remedios *et al.* 2015), ii) they are distributed throughout the world, iii) they include examples of both migrant and resident species, and iv) they display well-documented, highly variable mating system behaviours (Figure 1.1., Eberhart-Phillips *in press*, Thomas *et al.* 2007). Plovers have contributed substantially to the formulation of hypotheses linking mating system, dispersal and genetic structure. There is large interspecific variation in the breeding dispersal distances travelled by both sexes within plovers, for example, in the monogamous white fronted plover (*Charadrius marginatus*) the average distance travelled was less than 1 km (Lloyd, 2008), compared to an average of 145 km (maximum 1140 km) in the polyandrous snowy plover (Figure 1.2., *Charadrius nivosus*, Stenzel *et al.*, 1994). In Kentish plovers (*Charadrius alexandrinus*), mate change has been associated with long distance breeding dispersals (Székely and Lessells, 1993). Additionally, in a recent experimental mate replacement study, polygamous Kittlitz's plovers (*Charadrius pecuarius*) travelled further to find new mates than monogamous white fronted plovers (Cunningham *et al.* 2018).

The association between dispersal and mate changing may be more common than is documented but could easily remain undetected due to logistical constraints of monitoring multiple breeding populations simultaneously (Székely and Lessells 1993). Double clutching has been documented within polygamous and monogamous plovers (Lessells 1984, Warriner and Warriner 1978, Székely and Lessells 1993, Stenzel *et al.* 1994, Amat *et al.*, 1999, Andersson and Wallander, 2003, Yasué and Dearden 2008, Pearson and Colwell, 2014), however, there is currently no empirical evidence for a link between mate change, long distance breeding dispersal, and increased reproductive success. Rather, mate changing and double clutching have instead been associated with previous breeding success (e.g. Flynn *et al.* 1999, Skrade and Dinsmore 2010, Pearson

and Colwell 2014, Rioux *et al.* 2011), death of a mate (e.g. Friedrich *et al.* 2015) and environmental conditions (Stenzel *et al.* 1994).

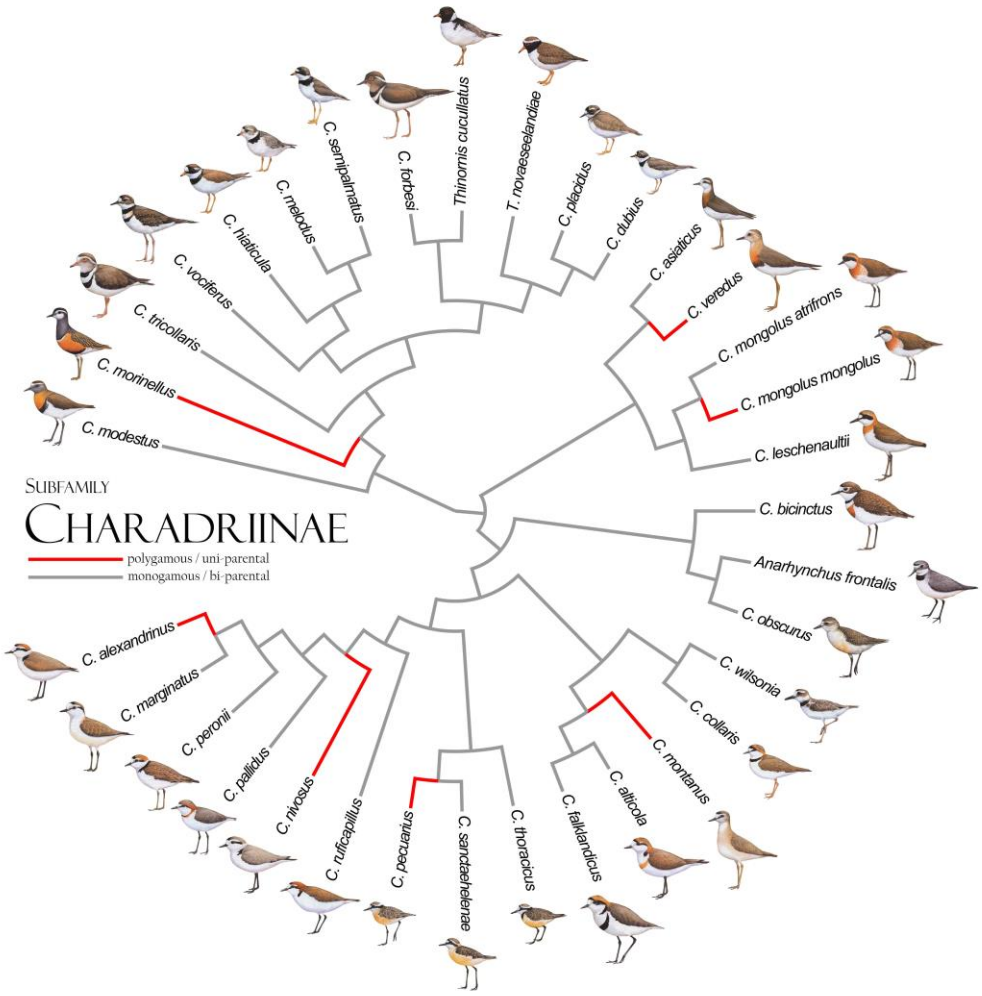

Figure 1.1. Cladogram of plover species (subfamily Charadriinae) and their diversity of mating systems. Grey branches = monogamous, red branches = polygamous. Reproduced with permissions from Eberhart-Phillips (*in press*). Illustrations from del Hoyo *et al.* (1996), phylogeny based on dos Remedios *et al.* (2015), graphical layout designed by Luke Eberhart-Phillips.

Figure 1.2. Snowy plover *Charadrius nivosus* adult with chick. Photo credit to Florida Fish and Wildlife Conservation Commission. Photo by Penny Jarrett, downloaded from https://creativecommons.org/licenses/by-nd/2.0/

Snowy plover

Until molecular and morphometric analysis was conducted in 2009 (Küpper *et al.* 2009), snowy plovers were considered part of the Kentish plover species complex. Snowy plovers are partially migratory and their populations are divided into three subspecies which span the Americas (American Ornithologists' Union 1957, Funk *et al.* 2007, Page *et al.* 2009). As a ground nesting species that inhabit the salt flats of alkaline lakes, coastal lagoons and sandy beaches, snowy plovers often suffer from disturbance and habitat destruction which impedes the persistence of some populations (Colwell *et al.* 2007a, Küpper *et al.* 2011, Powell and Collier 2011, Thomas *et al.* 2012, Cohen *et al.* 2014, Galindo-Espinosa and Palacios 2015, Cruz-López *et al.* 2017). The species as a whole is considered "near threatened" according to the IUCN (BirdLife International 2017), however, specific populations have endangered species protection under federal and state laws (Haig *et al.* 2011, Cohen *et al.* 2014, Galindo-Espinosa and Palacios 2015). As a classically polyandrous species, snowy plovers exhibit uniparental, male only care and females frequently breed sequentially with more than one mate per breeding season (Warriner *et al.* 1986, Eberhart-Phillips *et al.* 2017). Snowy plovers are one of few shorebirds with individual movement (Stenzel *et al.* 1994, 2007, Colwell *et al.* 2007b, Pearson and Colwell 2014) and genetic (Funk *et al.* 2007) data available. However, genetic analyses was restricted to few mtDNA and autosomal markers (Funk *et al.* 2007). Given the high dispersal ability of this species, it is possible that this study did not have the informative power to detect fine scale genetic patterns. A comprehensive genetic re-examination of snowy plovers is necessary to provide well informed estimates of genetic connectivity and diversity across the range. Furthermore, using sex specific genetic markers will improve our understanding of the genetic consequences of polyandry.

Jacanas (Family: Jacanidae)

"…as objects of interest, with outlandish ways and marvellous adaptations for coping with their watery world, [African jacanas] deserve their place among the world's more unusual birds." **W. Tarboton**. The Bird that Walks on Water

There are eight extant species of jacana, all of which are spread throughout the world's tropical and subtropical wetlands (Jenni 1996). With the exception of one species (lesser jacana, *Microparra capensis*), jacanas are characterised by classical simultaneous polyandry with sex-role reversal (Jenni and Collier 1972, Vernon 1973, Tarboton and Fry 1986, Thong-aree *et al.* 1995, Butchart 2000, Mace 2000, Emlen *et al.* 2004). However, this mating system can only be inferred for the Madagascar jacana (*Actophilornis albinucha*, Figure 1.3, Safford 2013) which has so far been neglected in behavioural and ecological studies.

Figure 1.3. Madagascar jacana, *Actophilornis albinucha* male (left) and female (right).

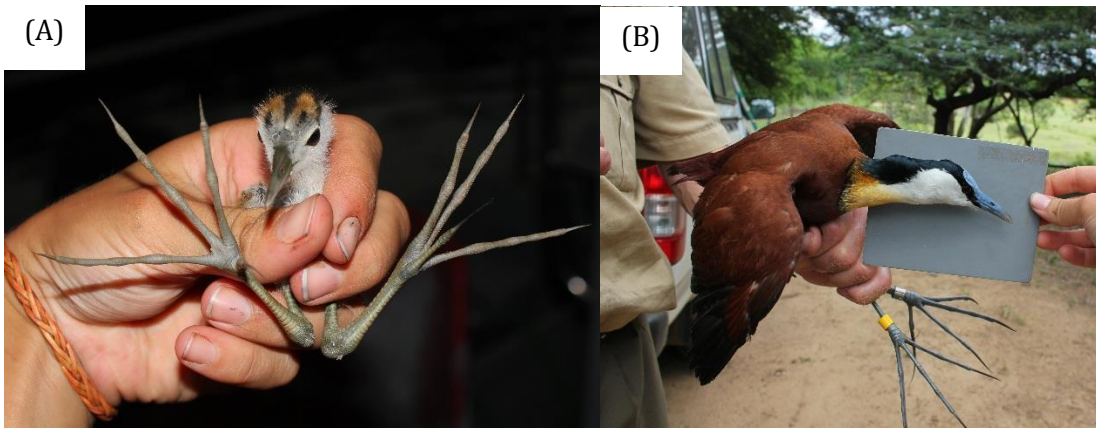

Figure 1.4. African jacana *Actophilornis africanus.* (A) chick (B) adult female with colour rings.

This charismatic group of birds play a vital role in our understanding of mating system evolution, specifically of female emancipation (Emlen *et al.* 2004). The high clutch loss rate, in addition to the rich productivity of tropical wetlands have been inferred to have promoted the female's ability to produce multiple clutches (Tarboton 1995, Butchart 2000). Combined with the low energetic costs of parental care in hot and humid habitats and extremely low clutch mass to female mass ratio, jacanas support the "easy breeding" hypothesis for the evolution of classical polyandry (Maxson and Oring 1980, Tarboton 1995, Bonkewitzz 1997).

Actophilornis jacanas encompass two out of three Old world jacana species the African jacana (*Actophilornis africanus,* Figure 1.4.) and the Madagascar jacana which are endemic to sub-Saharan Africa and Madagascar jacana respectively. The African jacana has the largest range of all jacana species (Jenni 1996, Tarboton 2005), and by taking advantage of man-made wetlands e.g. sewage works, this species has expanded in recent

years (Okes *et al.* 2008). African jacana are one of the continent's most iconic wetland animals and their polyandrous breeding system has been well studied in South Africa using long term datasets (Tarboton 1992a, b, 1993, 1995, 1996, Bonkewitzz 1997). They can breed all year round (Jenni 1996, Tarboton 2005), however, in South Africa the majority of nests are laid between November and February (Tarboton 1995), coinciding with increased rainfall. African jacana are highly polyandrous, with an extreme ability for rapid multi clutching, for example, one female was witnessed laying 10 clutches for up to six males per season (Tarboton 1992b). Local environmental conditions can also influence the degree of polyandry in African jacanas, with periods of drought associated with reduced instances of polyandry (Tarboton 1995).

The Madagascar jacana is one of 18 endemic wetland birds in Madagascar and is phenotypically very similar to the African jacana, although with slightly modified neck plumage (Madagascar jacana: Figure 1.3., African jacana: Figure 1.4.). The range of the Madagascar jacana is predicted to stretch along the west of Madagascar and into the Northern tip of the country, however, many of the records that this is based on are out of date (Safford 2013). Due to a predicted declining population trend and continued threats to freshwater wetlands in Madagascar, in 2016 the IUCN threat status of the Madagascar jacana was elevated to "near threatened" (BirdLife International 2016). However, the data quality of the Madagascar jacana population size is "poor" and the limited data available for the species hinders conservation efforts (BirdLife International 2016). Much of our understanding of the Madagascar jacana, including their mating system stems from anecdotal accounts or has been inferred from the African jacana (Jenni 1996, Safford 2013).

It is likely that these two species have only recently diverged based on their phenotypic similarity, and close geographic proximity, although no genetic phylogeny has included both species (Whittingham *et al.* 2000). New world jacanas, *Jacana,* are the only other jacana genus that include more than one species, however, the range overlap and occurrence of hybridisation between these species (Miller *et al.* 2014) adds additional complexities to interpreting their genomic architecture. Consequently, *Actophilornis* jacanas provide a rare opportunity to study the consequences of classical polyandry at progressive evolutionary scales, from within a population to the between the species.

Overview

This dissertation aims to understand micro- and macro-evolutionary consequences of mating systems and dispersal. Whilst mating systems, dispersal and genetic diversity have been investigated in a pair-wise manner, a novel aspect of my dissertation is that I take an integrative approach and seek to establish the multi-way interactions between these processes. Importantly, I also evaluate the significance of these processes for biodiversity conservation.

I begin with a meta-analysis to compare broad intraspecific patterns of the population differentiation of shorebirds exhibiting different mating systems (Chapter 2). I then increase the power of my methods by employing a combined genetic marker approach to detect fine-scale, sex specific structure in shorebirds that exhibit the rare mating system of classical polyandry (Chapter 3 and 4). I start by comprehensively reassessing the genetic diversity and differentiation in the snowy plover, a threatened and weakly polyandrous species (Chapter 3). I then explore the genetic consequences of more extreme classical polyandry demonstrated by simultaneously polyandrous and sex-role reversed *Actophilornis* jacanas (Chapter 4). In Chapter 5, I provide the first detailed ecological account of the understudied Madagascar jacana, to infer their distribution, evolutionary history and mating system. Finally, I synthesise my findings and discuss their implications to evolutionary and conservation biology (Chapter 6). My research aims to advance our understanding of the relationship between mating systems, dispersal and their population genetic consequences and in addition, to demonstrate how we can use this information to conserve biodiversity.

Objective 1. To investigate the relationships between mating systems, dispersal and diversification (Chapter 2).

In this chapter I test the hypothesis that polygamous species are characterised by reduced population differentiation compared to monogamous species, due to higher breeding dispersal to find multiple partners ("dispersal-to-mate" hypothesis). To do this I use two scales of data, intraspecific population genetic data from microsatellites from 10 plover species, and subspecies richness data from 136 shorebird species.

Objective 2. To reassess the genetic diversity and population structure of the sequentially polyandrous snowy plover, *Charadrius nivosus* (Chapter 3)

In this chapter I focus on the Snowy plover to conduct a thorough re-evaluation of the genetic population structure, ten years after the previous assessment. I use a combination of mtDNA, Z-linked SNPs and autosomal SNPs to determine the genetic support for subspecies. In addition I test an assumption of the "dispersal-to-mate" hypothesis from Chapter 2, that gene-flow will be female biased in this species because snowy plovers are classically polyandrous. Snowy plovers were the only polygamous species in Chapter 2 to show signals of population structure, which could be the result of barriers to gene-flow or demographic changes, therefore, I additionally assess signals of population bottlenecks and expansions. In the supplementary material of this chapter I tested the hypothesis that classical polyandry, which is associated with a male biased adult sex ratio and a skew in female reproductive success, will lead to higher Z chromosome diversity compared to autosomal diversity.

Objective 3. To characterise the genomic signature of simultaneous polyandry in *Actophilornis* jacanas (Chapter 4).

In this chapter, I extend my investigation into the genomic signatures of classical polyandry by investigating two jacana species that are known, or expected to, exhibit extreme polyandry. I used a hierarchical approach to assess the genetic divergence and differentiation on the sex chromosomes, between the populations, and also between the species with mtDNA and RADseq data. As in Chapter 3, I hypothesised that polyandry would be associated with high Z chromosome diversity, but additionally tested if this high diversity reduced intra- and inter-specific divergence by reducing the power of genetic drift. However, as high Z chromosome diversity can also result from demographic expansions, I also assessed whether either species had experienced population increases or decreases. In addition, I continued to test the "dispersal-to-mate" hypothesis that polyandrous species are characterised by female biased dispersal.

Objective 4. To describe the ecology, distribution and threat status of the endemic Madagascar jacana, *Actophilornis albinucha* (Chapter 5)

In my final data chapter, I conducted an ecological assessment of the Madagascar jacana, which is an understudied species that is predicted to exhibit classical polyandry and sex-role reversal. Madagascar wetlands are highly threatened and we risk losing species without having adequate estimates of their basic biology, population size and distribution. In this chapter I used field surveys to determine the current distribution and population density, in addition to estimating the threats they face. Sexual size dimorphism (SSD) can be associated with mating system, therefore, I compared male and female morphometric measurements to estimate the direction of SSD, with the hypothesis that, females would be significantly larger than males. Finally, I assessed their evolutionary relationships by creating the first genetic phylogeny including all extant jacana species.

This thesis is presented in the alternative thesis format, with each data chapter including supplementary material and a commentary before and after.

Tables and figures in the main text are presented as: Figure/Table {chapter number}.X
Supplementary tables and figures are presented as: Figure/Table S{chapter number}.X
Commentary tables and figures are presented as Figure/Table C{chapter number}.X.

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Appendix B: Statement of Authorship

<table>
<tr><th colspan="10">This declaration concerns the article entitled:</th></tr>
<tr><td colspan="10">Polygamy slows down population divergence in shorebirds</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td></td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td>X</td></tr>
<tr><td>Publication details (reference)</td><td colspan="9">Josephine D'Urban Jackson, Natalie dos Remedios, Kathryn H. Maher, Sama Zefania, Susan Haig, Sara Oyler-McCance, Donald Blomqvist, Terry Burke, Michael W. Bruford, Tamás Székely and Clemens Küpper (2017) Polygamy slows down population divergence in shorebirds Evolution 71: 1313-1326</td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">J.D.J. designed methodology with C.K., T.S. and M.W.B. Samples and genotypes were not collected by J.D.J. J.D.J executed all population genetic data analyses and wrote the first draft of the manuscript. The manuscript was improved further by co-authors and two anonymous reviewers. Manuscript alterations were conducted by J.D.J All figures were created by J.D.J.
J.D.J. contribution 80%</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
<tr><td>Signed</td><td colspan="6"></td><td>Date</td><td colspan="2"></td></tr>
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Pre Chapter 2 Commentary

This chapter aims to provide a basis to my thesis by investigating if sexual selection and/or dispersal capacity predict diversification. By testing for broad patterns across and within species I was able to refine my hypotheses for the following chapters and identify areas which require additional investigation.

I used several proxy measures to do this. Polygamous species represented those under high sexual selection pressure compared to a monogamous species, and migratory species represented those with higher dispersal capacity compared to non-migratory species. Diversification was assessed at two scales, firstly, intraspecific spatial genetic patterns from 10 plover species (Genus: *Charadrius*), and secondly, subspecies richness from 136 shorebird species (Family: Charadriiformes). I introduced and tested the "dispersal-to-mate" hypothesis, which predicts that polygamous species are characterised by reduced population structure and fewer subspecies compared to monogamous species. Additionally, I predicted that high dispersal capacity is associated with weaker genetic structure compared to lower dispersal capacity.

2

Polygamy slows down population divergence in shorebirds

Josephine D'Urban Jackson, Natalie dos Remedios, Kathryn H. Maher, Sama Zefania, Susan Haig, Sara Oyler-McCance, Donald Blomqvist, Terry Burke, Michael W. Bruford, Tamás Székely and Clemens Küpper

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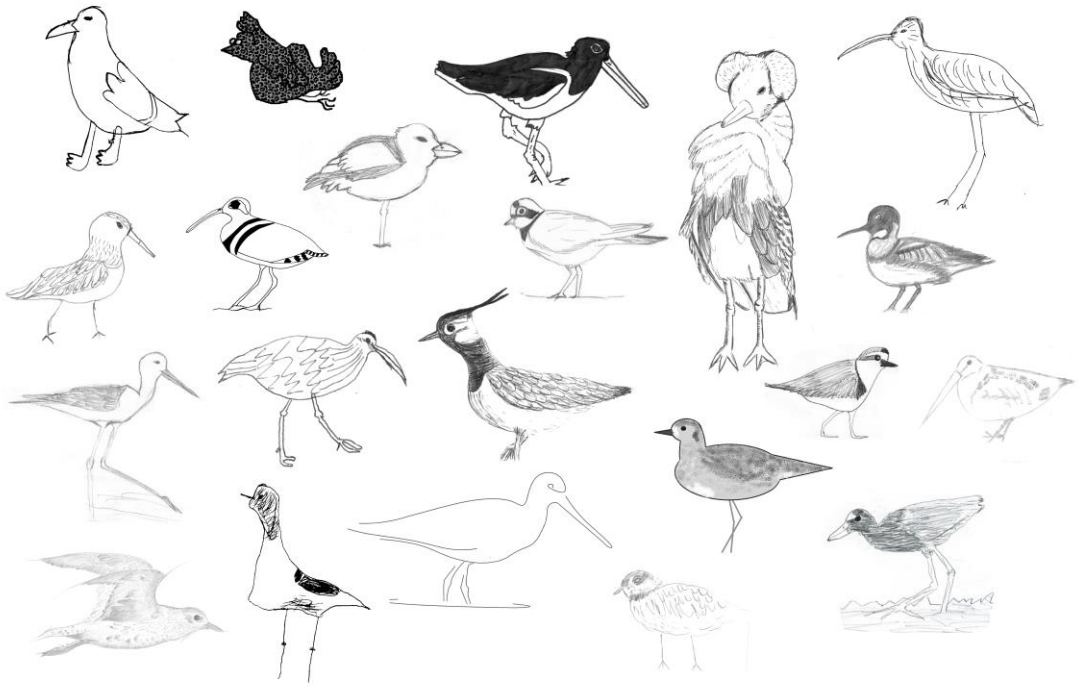

Shorebirds by family, friends, and colleagues aged 7 – 50-something years old

"…to watch the flight of shore birds that have swept up and down the surf lines of the continents for untold thousands of years, to see the running of the old eels and the young shad to the sea, is to have knowledge of things that are as nearly eternal as any earthly life can be." ***Rachel Carson*** *(1955) The Edge of The Sea*

Author contributions

J.D.J. performed the analysis and prepared the manuscript
N.d.R, K.H.M., S.Z. S.O-M., S.H., D.B. and T.B. provided samples and/or microsatellite genotypes; manuscript improvement
C.K. and T.S. idea conception and design; advice on methods; manuscript improvement
M.W.B. advice on methods; manuscript improvement

Polygamy slows down population divergence in shorebirds

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Abstract

Sexual selection may act as a promotor of speciation since divergent mate choice and competition for mates can rapidly lead to reproductive isolation. Alternatively, sexual selection may also retard speciation since polygamous individuals can access additional mates by increased breeding dispersal. High breeding dispersal should hence increase gene-flow and reduce diversification in polygamous species. Here we test how polygamy predicts diversification in shorebirds using genetic differentiation and subspecies richness as proxies for population divergence. Examining microsatellite data from 79 populations in ten plover species (Genus: *Charadrius*) we found that polygamous species display significantly less genetic structure and weaker isolation-by-distance effects than monogamous species. Consistent with this result, a comparative analysis including 136 shorebird species showed significantly fewer subspecies for polygamous than for monogamous species. By contrast, migratory behaviour neither predicted genetic differentiation nor subspecies richness. Taken together, our results suggest that dispersal associated with polygamy may facilitate gene-flow and limit population divergence. Therefore, intense sexual selection, as occurs in polygamous species, may act as a brake rather than an engine of speciation in shorebirds. We discuss alternative explanations for these results and call for further studies to understand the relationships between sexual selection, dispersal and diversification.

Keywords: Mating systems, sexual selection, dispersal, shorebird, gene-flow, speciation, migration

Introduction

Sexual selection is often thought of as a facilitator of speciation via female mate preferences leading to prezygotic reproductive isolation (the "engine of speciation" hypothesis; Morrow *et al.* 2003). Intense sexual selection can lead to rapid speciation in at least four different ways (Ritchie 2007, Wilkinson and Birge 2010, Gavrilets 2014). First, female preference for males that exhibit particular traits may lead to coevolution between males exhibiting the traits and females preferring the trait either via selection for good genes or sexy sons (Fisher 1930, Lande 1981, Kirkpatrick 1982, West-Eberhard 1983, Fowler-Finn and Rodríguez 2016, Ellis and Oakley 2016). Second, negative frequency dependent selection on sexually selected traits that are important during intrasexual competition may ultimately result in reproductive isolation. (Greene *et al.* 2000, Seehausen and Schluter 2004, Clutton-Brock and Huchard 2013). Third, sexual selection might be associated with ecological speciation where sexually selected traits or those involved in sexual communication are under divergent natural selection (Maan and Seehausen 2011, Safran *et al.* 2013). Fourth, sexually antagonistic coevolution, termed sexual conflict (Parker 1979), between males and females may drive an arms race with male and female (counter) adaptations that lead to exaggerated traits which then form reproductive barriers (Gavrilets 2014).

By contrast, sexual selection may also reduce, instead of amplify, reproductive isolation between populations under some evolutionary scenarios. For example, sexual conflict may enhance interpopulation gene-flow if female resistance to pre- and post-mating manipulation prevents matings in some populations, therefore, promoting the dispersal of local males to find naïve females that have not developed counteradaptations in neighbouring populations (Parker and Partridge 1998). In addition, sexual selection could also limit sympatric speciation as assortative mating can reduce the variation that could be selected upon leading to the fixation of certain traits (Kirkpatrick and Nuismer 2004).

Variance in mating success is typically larger in polygamous than in monogamous species. Polygamous individuals attempt to access as many mates as possible and may need to disperse, especially when breeding is highly synchronised locally, to maximise their reproductive success. Dispersal to increase mate access has been suggested to explain why adults of polygamous and promiscuous birds and mammals travel large distances during the breeding season (Blundell *et al.* 2002, Woolfenden *et al.* 2005, Debeffe *et al.* 2014, García-Navas *et al.* 2015, Davidian *et al.* 2016, Kempenaers and Valcu 2017), whereas monogamous species are often more faithful to breeding sites (Pitelka *et al.* 1974, Saalfeld and Lanctot 2015). High breeding dispersal is likely to lead to low levels of genetic differentiation within a polygamous species (Küpper *et al.* 2012, Verkuil *et al.* 2012, Eberhart-Phillips *et al.* 2015). This gene-flow may prevent reproductive isolation by counteracting the effect of processes such as genetic drift and local adaptation and thus slowing speciation processes (here termed the "dispersal-to-mate" hypothesis).

Regular migration movements outside the breeding season may also influence diversification (Phillimore *et al.* 2006, Garant *et al.* 2007, Weeks and Claramunt 2014, Arendt 2015). Intuitively, high dispersal abilities should reduce genetic differentiation between populations (Belliure *et al.* 2000, Garant *et al.* 2007, Claramunt *et al.* 2012,

Weeks and Claramunt 2014). Indeed, many examples of low genetic differentiation among breeding populations of migratory species are found in birds and mammals (e.g. Webster *et al.* 2002, Friesen *et al.* 2007, Burns and Broders 2014). However, high (and leptokurtic) dispersal can also lead to the colonisation of remote areas such as oceanic islands that are too far away from the core population to maintain regular gene-flow. After the colonization event, local adaptation and genetic drift in combination with behavioural changes may then lead to allopatric differentiation (Rosenzweig 1995, Owens *et al.* 1999, Phillimore *et al.* 2006). Corroborating this hypothesis, seasonal migration has been linked to greater net diversification rates in birds where colonisation events are followed by settling down and loss of annual migratory behaviour (Rolland *et al.* 2014).

Shorebirds (sandpipers, plovers and allies; *Scolopaci, Thinocori, Chionidi* and *Charadrii*) are a diverse and ecologically well-characterised avian clade which display huge variation in mating systems and migratory behaviour (Székely *et al.* 2000, Piersma and Lindström 2004, Thomas *et al.* 2007, García-Peña *et al.* 2009). This group of taxa therefore provide an ideal opportunity to investigate the relationship between mating systems, migratory behaviour and diversification. The objectives of our study were to test whether polygamous species that are under high pressure to access multiple mates, and thus are subject to strong sexual selection, showed higher diversification than monogamous species, as predicted by the "engine-of-speciation" hypothesis or lower diversification consistent with the "dispersal-to-mate" hypothesis. Mating systems have a significant influence on the variation of individual mate success, with polygamy leading to greater variation in mating success across individuals compared to monogamy (Emlen and Oring 1977, Shuster and Wade 2003). For this reason we used mating system as a proxy for strength of sexual selection as we hypothesised that due to this high variation in breeding success, polygamous individuals move between breeding populations in an attempt to elevate their chance of successful matings (Breiehagen 1989, Székely and Lessells 1993, Stenzel *et al.* 1994, Kempenaers and Valcu 2017).

We investigated the relationships between mating systems, migration and diversification using two data sets with either genetic differentiation or subspecies richness as proxy for within species population divergence and hence speciation propensity. Firstly, we studied plovers (*Charadrius* spp.), a globally distributed clade of shorebirds that includes both migrant and resident species with monogamous or sequentially polygamous mating systems (Thomas *et al.* 2007, dos Remedios *et al.* 2015). Within a breeding season sequentially polygamous plovers change partners after a successful breeding attempt, leaving their mate to care for the young, whereas, monogamous plovers stay together for subsequent breeding attempts. Social mating system reflects genetic mating system in plovers, since extra-pair paternity is rare in these species (less than 5 %, Maher *et al.* 2017). Using ten *Charadrius* species we tested whether intraspecific patterns of genetic differentiation were associated with mating system and/or migratory behaviour using microsatellite datasets. Secondly, since similar genetic data are only available for a fraction of shorebirds, we expanded our analyses to include 136 shorebird species and test whether mating system and/or migratory behaviour predicted subspecies richness, an alternative measure for diversification (Belliure *et al.* 2000, Phillimore and Owens, 2006, Martin and Tewksbury, 2008).

Materials and methods

Genetic differentiation in plover populations

We analysed published and newly collected microsatellite data from ten plover species (Genus: *Charadrius*): Kittlitz's plover (*C. pecuarius*; Eberhart-Phillips *et al.* 2015, dos Remedios 2013), Madagascar plover (*C. thoracicus*; Eberhart-Phillips *et al.* 2015), white-fronted plover (*C. marginatus*; Eberhart-Phillips *et al.* 2015, dos Remedios 2013), chestnut-banded plover (*C. pallidus*; dos Remedios et al. 2017), Kentish plover (*C. alexandrinus*; Küpper *et al.* 2012), mountain plover (*C. montanus*; Oyler-McCance *et al.* 2008) and piping plover (*C. melodus*; Miller *et al.* 2010). In addition, further plover populations from three species were genotyped including, snowy plover (*C. nivosus*), common ringed plover (*C. hiaticula*) and killdeer (*C. vociferous*). Sampling locations were distributed across all continents except Australia, South America and Antarctica (Table 2.1 is presented after the references; Figure 2.1.) and included four resident and six migratory species with different mating systems (six monogamous and four polygamous) and wide variation in breeding range sizes (Table 2.1). The detection of spatial genetic pattern can be highly sensitive to factors such as the number of loci and the number of alleles per locus (Landguth *et al.* 2012), however, across the datasets we found no relationship between the number of loci or the average number of alleles per locus and the detection of spatial genetic patterns (see Supplementary material). For microsatellite marker characteristics and laboratory protocols see Table S2.1.

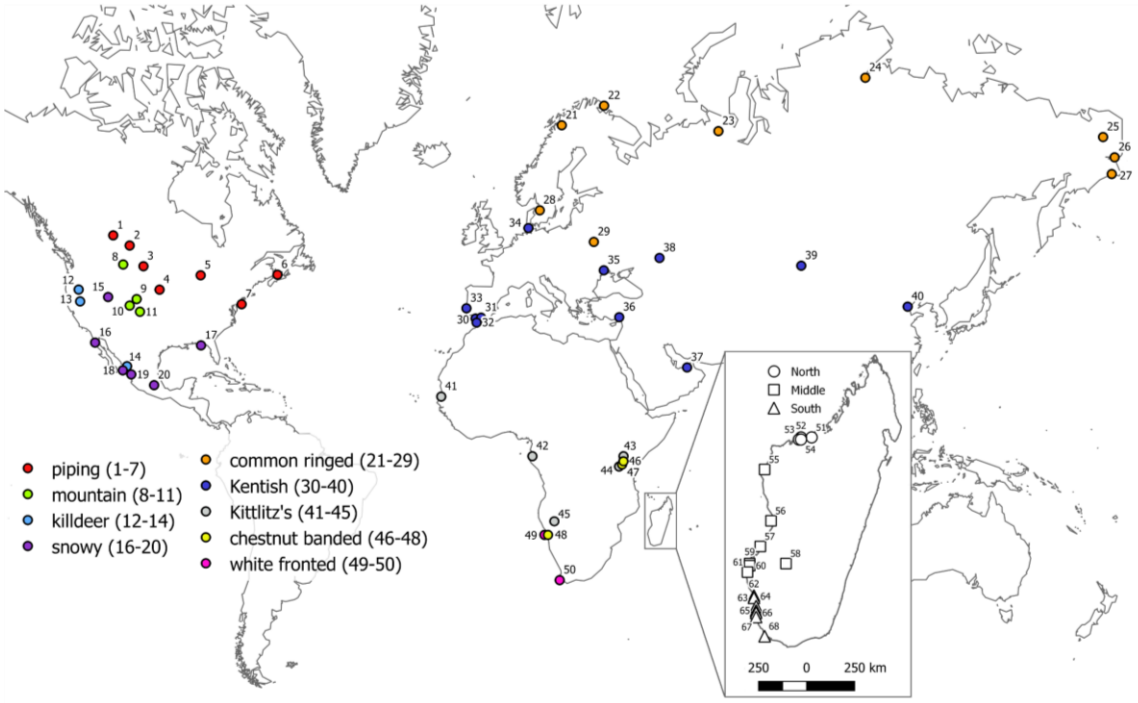

Figure 2.1. Sampling locations of *Charadrius* plover populations for genetic differentiation analyses. Numbers refer to population information (Table 2.1). In Madagascar insert, symbols do not represent species but rather they show position of sites in North, Middle and South Madagascar.

Due to potential bias of null alleles during the estimation of population subdivision (F_{ST}) and genetic distance (Chapuis and Estoup 2007; Dabrowski *et al.* 2014), null allele frequencies and genotyping errors were estimated for all data using Microchecker v2.2.3 (Van Oosterhout *et al.* 2004). Loci identified as having null alleles in the majority of the populations were removed for Bayesian clustering analysis, and pairwise F_{ST} values were corrected for the presence of null alleles using FreeNA (Chapius and Estoup 2007). Individuals with more than 15% missing data were excluded from further analyses.

We used a Bayesian clustering algorithm implemented in STRUCTURE v2.3.4 (Pritchard *et al.* 2000) to determine the extent of population structure within each species. We used the admixture model with location information as a prior, an approach that is required when structure is expected to be weak (Pritchard *et al.* 2000, Hubisz *et al.* 2009). This approach improves cluster outcomes by favouring the clustering of individuals that were sampled together. However, it is worth noting that this method does not detect structure if there is none (Pritchard *et al.* 2000, Hubisz *et al.* 2009). Location priors for each population are provided in Table 2.1 For breeding locations with less than 10 samples we ran the analysis twice, first giving them unique location priors and again after removing these populations. All analyses were run with a burn-in period of 100,000 followed by 1,000,000 Monte Carlo Markov Chain (MCMC) repeats for ten replicates. Initially, the number of clusters tested were between one and the maximum number of locations sampled (Table 2.1). We then summarised the results with STRUCTURE HARVESTER v0.6.94 (Earl and VonHoldt 2012) and estimated the most likely number of clusters present based on likelihood and Delta K (Evanno *et al.* 2005). Bar plots representing admixture proportions for the most likely *K* values were examined to assess whether the results of Delta K and likelihood methods were biologically meaningful. Individual admixture proportion information was merged from the ten repeats using the "full search" method in CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007). If the initial best model suggested $K \geq 2$ and the admixture proportions of individuals within these populations was less than 0.01, the data set was split into the identified clusters and we repeated the Bayesian clustering until the best model in STRUCTURE was $K = 1$, similar to progressive partitioning (Hobbs *et al.* 2011).

The number of clusters identified by STRUCTURE were compared for species with different mating systems ('monogamous' or 'polygamous') and migratory behaviour ('resident' or 'migratory'). Sea distance is an effective barrier of gene-flow in plovers (Küpper *et al.* 2012). For species distributed and sampled on more than one land mass, we included only the data set with the largest number of samples and locations. Species were assigned to categories 'one' or 'more than one genetic cluster' and we compared frequencies to expected 1:1 values using Fisher's exact tests (Fisher 1922).

We used the scoring system based on Thomas *et al.* (2007) to classify the mating system of each species (Székely *et al.* 2004, García-Peña *et al.* 2009, Olson *et al.* 2009) and updated the mating system information for species with new data (Table S2.2 and S2.3). However, we simplified the scoring for the purpose of this study using only two instead of five categories, since only a few dispersers per generation are required to maintain gene-flow (Spieth 1974, Mills and Allendorf 1996). We classified the categories 0 and 1 of Thomas *et al.* (2007), which correspond to ≤1% polygamy in either sex observed during breeding behaviour studies, as 'monogamous' and groups 2-4 (for species that

are known to display more than 1 % polygamy in either sex) as 'polygamous'. Migratory status was classified as either 'migrant' (including partially migrant species) or resident. Migratory information was collected from Bird Life International (http://www.birdlife.org/datazone/species, accessed: January 2017) (Tables S2.2 and S2.3).

To examine the degree of isolation-by-distance (IBD) for the ten plover species we performed Mantel Tests (Mantel 1967, Mantel and Valand 1970) using a population based approach instead of alternative landscape genetic approaches (e.g. multiple regression analysis) since individual location and environmental data were not available for all species. We calculated Euclidean distance matrices between populations using GenALEx v6.501 (Peakall and Smouse 2012). Using log-transformed geographic distances (Legendre and Fortin 2010) provided the same qualitative results (results not shown). We calculated pairwise Rousset's linearised F_{ST} ('F_{ST}' hereafter) from the null allele corrected F_{ST} values, using the following equation: $F_{ST} / (1- F_{ST})$ (Rousset 1997). All Mantel tests were performed with the package 'adegenet' (Jombart 2008).

To test whether mating system and/or migratory status affects spatial genetic patterns, we used the slope of a linear regression line between genetic (F_{ST}) and geographic distance for each species as a proxy for the strength of IBD ('IBD gradient' hereafter). This was calculated because of potential bias involved in directly comparing average F_{ST} values between species due to the ascertainment biases of microsatellite markers, since 75% of the markers used were developed for one species specifically (Küpper *et al.* 2007).

Following tests for normality of the IBD gradient, we performed phylogenetic least squares analysis (PGLS; Freckleton *et al.* 2002) to account for phylogenetic autocorrelation between species using the 'caper' package (Orme 2013), (in addition to generalised linear models (GLM) with Gaussian errors) to examine the influence of mating system and/or migratory behaviour on the IBD gradient using 'species' as the statistical unit. The recently published *Charadrius* phylogeny (dos Remedios *et al.* 2015) was used to measure phylogenetic relatedness between species for the PGLS analysis. Species with large breeding range sizes are likely to have greater levels of differentiation between populations compared to those with smaller ranges (Gavrilets and Vose 2005; Losos and Parent 2009; Kisel and Barraclough 2010), therefore we incorporated breeding range size into the models. Due to large differences between species breeding range sizes, which may influence the IBD gradient, log breeding range size was included in the model. As our sample size is small (n=10) we fitted and compared single parameter models to avoid overfitting of models that may lead to inflation of statistical significance (Harrell 2001). The best fitting model was selected using an information theoretic approach (Burnham and Anderson 2002). This method ranks the models based on Akaike information criterion corrected for small sample sizes (AICc) and we assessed support for each model based on the differences in AICc (Δ_i) and Akaike weights (w_i) (Burnham and Anderson 2002). Substantial support for a model is indicated by Δ_i- values of less than 2 and of these, highly optimal models will have w_i values of more than 0.9 (Burnham and Anderson 2002). Model selection was performed using the 'MuMIn' package (Bartoń 2016).

Subspecies richness in shorebirds

To test our hypotheses that 1) polygamy restricts diversification and 2) migration restricts diversification, we used the subspecies richness of shorebird species (Order: Charadriidae; suborders: Charadrii, Chionidi, Scolopaci and Thinocori) as a proxy for the degree of diversification. This allowed us to test for drivers of diversification in a much larger data set. Avian subspecies richness has been used as a proxy for population differentiation in previous studies testing the drivers of diversification (Belliure *et al.* 2000, Phillimore and Owens 2006, Martin and Tewksbury 2008). We used subspecies information from the IOC World Bird List v 7.1 (Gill and Donsker 2016). This database is updated annually with new information from peer reviewed articles. Subspecies delineations are not always supported by genetic data (Phillimore and Owens 2006), however, in absence of genetic data these delineations provide a useful proxy for diversification in comparative studies at lower taxonomic levels. We classified mating systems and migratory status using the same methods as in the plover analyses above (Tables S2.2 and S2.3). We again performed PGLS analysis, and in addition to mating system and migratory status we also included log breeding range size. Shorebirds without mating system information or with only anecdotal evidence of mating system category were excluded, as were species without breeding range size data.

We selected 100 phylogenetic trees at random using the phylogeny of Jetz *et al.* (2012), downloaded from http://birdtree.org (accessed in: December 2016). We repeated the analysis using both Hackett *et al.* (2008) and Ericson *et al.* (2006) phylogenetic backbones and no differences were found between the methods. We removed four species (*C. nivosus, Coenocorypha huegeli, Nycticryphes semicollaris* and *Gallinago delicata*) from the analysis as they were not included in the Jetz *et al.* (2012) phylogeny. This resulted in a final dataset of 136 shorebirds species (Tables S2.2 and S2.3) consisting of 108 monogamous species, 28 polygamous species or 83 migrant species and 53 resident species.

PGLS analysis was repeated for each of the 100 trees and the original model formula was as follows:

Total number of subspecies ~ mating system + migratory status + migratory status * mating system + log10 breeding range size

We then simplified models removing the least significant variable in a stepwise manner. As with IBD gradient GLMs we assessed the model fit for all model combinations using Δ_i and w_i values (Burnham and Anderson 2002).

For all statistical analyses, unless stated otherwise, we used R version 3.3.2 (R Development Core Team 2016).

Results

Genetic differentiation in plovers

We identified one locus, *Calex14* with a high probability of having null alleles in the killdeer, this locus was excluded from further analyses in this species. The average number of alleles per locus indicated large variation in genetic diversity between species (mean = 6.4 ± 3.5 SD). No difference in the clustering outcome was found when removing populations with less than ten individuals (data available on request). Progressive partitioning increased piping plover clustering outcome from two to three, indicating that in addition to a split between the two subspecies (*C. m. circuminctus* and *C. m. melodus*), there is also a differentiation in *C. m. melodus* between the Canadian and U.S. American sampling sites (Figure 2.2B). Mating system but not migratory behaviour was associated with the number of genetic clusters across the ten species (Fisher's exact tests: mating system: $p = 0.033$; migratory status: $p = 1$). We found fewer clusters within polygamous (mean ± SD: 1.25 ± 0.5) than within monogamous species (2.33 ± 0.5). We did not detect any differentiation within three of the four polygamous species across their sampled breeding populations (Figure 2.2A), whereas we detected at least two genetic clusters within all six monogamous species, comprising two clusters in four species and more than two clusters in two species (Figure 2.2B). The white-fronted and Kittlitz's plover exhibited consistent patterns between Madagascar and the African mainland, i.e. we detected genetic structure among monogamous white-fronted plover populations but not among polygamous Kittlitz's plover populations within each land mass. To avoid pseudoreplication we included only the larger Madagascar data set for both species in the subsequent analyses.

Across all plovers IBD was weak (Figure 2.3 and Table 2.2). Three monogamous species, white-fronted plover, piping plover and the common ringed plover showed significant IBD (Monte Carlo test observation, $r = 0.397$, 0.749 and 0.28 respectively; $p = 0.05$, 0.02, 0.05 respectively; Table 2.2), whereas for all other species we did not detect a significant association. The best model to explain variation in IBD gradient among the ten plover species contained only 'mating system' as an explanatory variable (PGLS $w_i = 0.86$) and no other model had a $\Delta_i\text{-} \leq 2$. The model suggested that monogamous species have significantly higher IBD gradients than polygamous species (PGLS: df = 8, $t = -2.49$, $p = 0.05$). Neither breeding range size nor migratory status predicted IBD gradients in plovers. For full model results of the PGLS and the GLM analyses see Table S2.4.

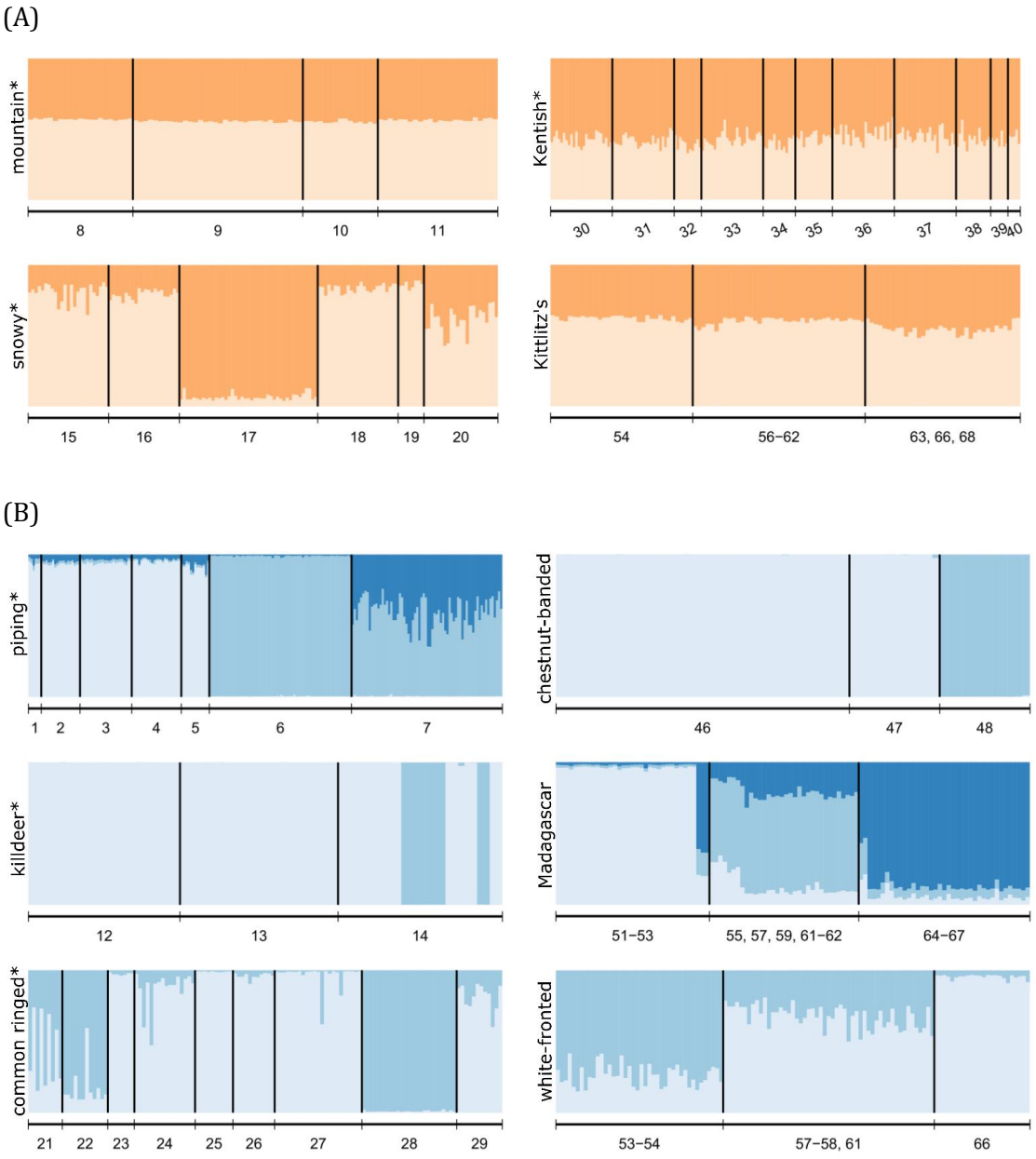

Figure 2.2. Bayesian population clustering of *Charadrius* plovers according to genetic differentiation in (A) polygamous, and (B) monogamous plover species. Migratory species are indicated by asterisk, otherwise a species is an all year resident. Each vertical line represents an individual, colours represent the membership proportion to a given genetic cluster. Models with two or three clusters are presented. See Table 2.1 for site ID number for each species.

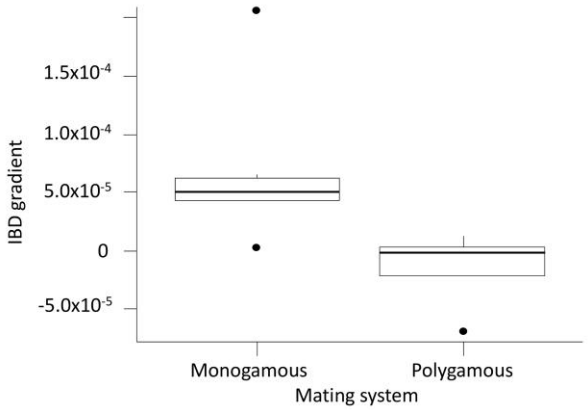

Figure 2.3. Isolation-by-distance (IBD) gradient of monogamous (n=6) and polygamous (n=4) *Charadrius* plovers.

Table 2.2. Patterns of isolation-by-distance across ten *Charadrius* plovers with different mating systems (P= polygamous; M= monogamous). Rousset's linearised F_{ST} was used as genetic distance in Mantel tests. *r* = Mantel test regression coefficient. Significant isolation-by-distance values ($p<0.05$) indicated with *

Plover species	Mating system	*r*	F_{ST} gradient
Kentish	P	0.19	7.15E-07
Kittlitz's	P	-0.28	-6.71E-05
mountain	P	0.74	1.37E-05
snowy	P	-0.10	-3.90E-06
white-fronted	M	0.40*	4.37E-05
Madagascar	M	0.16	4.60E-05
piping	M	0.76*	6.57E-05
common ringed	M	0.28*	3.86E-06
chestnut-banded	M	0.99	0.000208
killdeer	M	0.98	5.42E-05

Subspecies richness in shorebirds

Phylogenetic analysis in shorebirds showed that subspecies richness was best predicted by a model that included both mating system and breeding size range (Table S2.5). The minimal model indicated that monogamous species are divided into significantly more subspecies than polygamous species (Figure 2.4) and shorebirds with larger breeding ranges harboured more subspecies than small range species (PGLS model 3: df = 133 mating system t = -2.26, p = 0.026; log breeding range size t = 1.98, p = 0.05). Consistent with genetic results in plovers, migratory behaviour did not predict subspecies richness (PGLS model 2: df = 132, migratory behaviour t = -0.165, p = 0.896; Table S2.5).

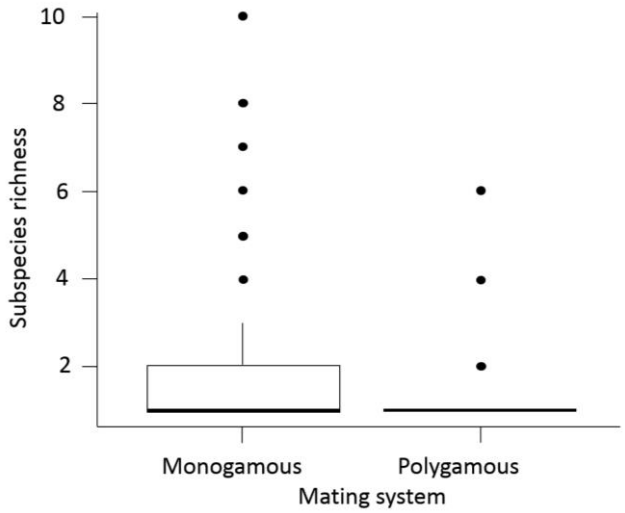

Figure 2.4. Subspecies richness of monogamous (n=108) and polygamous (n=28) shorebird species (Order: Charadriidae; suborders: Charadrii, Chionidi, Scolopaci and Thinocori).

Discussion

We investigated whether diversification in shorebirds is related to mating and/or migration behaviour using two complementary indices of population diversification: genetic differentiation in *Charadrius* plovers and subspecies richness across shorebird species. Consistent with previous studies (Møller and Cuervo 1998, Owens *et al.* 1999, Arnqvist *et al.* 2000) we found a relationship between mating system and diversification. However, contrary to previous suggestions that sexual selection facilitates speciation (West-Eberhard 1983, Panhuis *et al.* 2001, Ritchie 2007) we found that polygamous shorebird species (i.e., those with higher competition for mates), showed less genetic structure, weaker isolation-by-distance and lower subspecies richness compared to monogamous species. These results are consistent with the "dispersal-to-mate" hypothesis (i.e. intense sexual selection in polygamous species promotes breeding dispersal), which in turn leads to widespread gene-flow across the distribution range (Küpper *et al.* 2012). Our interpretations are supported by recent direct studies on breeding dispersal of polygamous sandpipers using satellite tag telemetry, where

lekking male pectoral sandpipers (*Calidris melanotos*) show exceptionally long distance breeding dispersal, moving more than 13,000 km during a single breeding season in search for new mating opportunities (Kempenaers and Valcu 2017). Similarly, in polygynous mammals polygynous males disperse between neighbouring populations, presumably to increase their access to oestrus females (Greenwood 1980, Olupot and Waser 2001) suggesting that the dispersal of the polygamous sex is influenced by the distribution of the opposite sex (Greenwood 1980).

Using genetic data from multiple shorebirds we show the evolutionary consequences of mating behaviour at the population level. Instead of promoting genetic isolation of populations, sexual selection rather seems to constrain speciation due to mate access pressure. The results on the genetic differentiation of plover populations were mirrored by our findings of subspecies richness across shorebirds that showed fewer subspecies in polygamous compared to monogamous shorebird species. Both data sets included polygynous and polyandrous taxa and hence sequentially polygamous males and females may be responsible for maintaining high gene-flow. Subspecies delineations are based often largely on divergent phenotypic characters and do not necessarily reflect findings on differentiation of neutral genetic markers (Phillimore and Owens 2006). Nevertheless, in our study, we found genetic support for all subspecies delineations within the plover species analysed (Table S2.6). Further, since subspecies definitions vary widely among authors and may not be supported by genetic data, subspecific delineation may in any case provide a complementary measure of ecological divergence that is then also associated with mating systems. Finally, subspecies richness may represent a more conservative measure for population differentiation than genetic differentiation since we found additional genetic structure *within* subspecies in the piping plover (*C. m. melodus*, Figure 2.2B), the common ringed plover (*C. h. hiaticula* and *C. h. tundra,* Figure 2.2A) and the snowy plover (*C. n. nivosus,* Figure 2.2A).

Our findings contribute to the debate concerning the role of sexual selection in speciation (Gage *et al.* 2002, Morrow *et al.* 2003, Kirkpatrick and Nuismer 2004, Maan and Seehausen 2011, Servedio and Kopp 2012, Servedio and Bürger 2014, Ellis and Oakley 2016). Previous studies have suggested at least five arguments to explain why sexual selection may not appear to promote diversification. Firstly, inconsistent results may emerge if both speciation and extinction rates are elevated in sexually selected species, and these two processes counterbalance each other (Morrow *et al.* 2003, but see: Morrow and Fricke 2004). Secondly, different mating systems may evolve between species after speciation has occurred (e.g. some clades may be more likely to develop certain breeding behaviour than others) and thus sexual selection is independent of speciation due to other mechanisms (e.g., local adaptation (Gage *et al.* 2002)). Thirdly, sexual selection may play a part in speciation, but mate preference alone may not be strong enough to prompt complete reproductive isolation (van Doorn *et al.* 2004, Servedio 2011, Servedio and Kopp 2012, Servedio and Bürger 2014). Fourthly, the effects of ecological speciation may mask the influence of sexual selection and these two forces could work antagonistically or together in speciation processes (Kraaijeveld *et al.* 2011, Maan and Seehausen 2011, Wagner *et al.* 2012). Finally, these inconsistent findings may in part be due to differences in methodologies used to investigate the relationship between sexual selection and speciation (Kraaijeveld *et al.* 2011).

Here we provide a hypothesis which emphasises that dispersal driven by mate access pressure needs to be taken into account in discussions concerning the importance of sexual selection in diversification processes. According to the "dispersal-to-mate" hypothesis, polygamous adults (polyandrous females or polygynous males) looking for new mates may often disperse to increase their pool of potential mates. When polygamous individuals reproduce at several sites they become a major contributor to high gene-flow. Field data suggest that male polygamous sandpipers disperse large distances during the breeding season (Kempenaers and Valcu 2017) and similarly, female polyandrous plovers tend to exhibit larger scale movements than males (Székely and Lessells 1993, Stenzel *et al.* 1994). These differences will ultimately be reflected in population genetic patterns. Consistent with female biased dispersal, maternally inherited mtDNA is less structured, whereas the Z-chromosomal DNA is more structured than autosomal microsatellites in the polyandrous Kentish plover (Küpper *et al.* 2012). However, the latter result may also reflect typical sex-specific natal dispersal patterns where female birds disperse more than males (Greenwood 1980, but see Mabry *et al.* 2013).

Natal dispersal may chiefly serve to avoid inbreeding but it has been also been linked to the mating system (Greenwood 1980). Sex-biased dispersal in birds and mammals may be related to either resource defence (birds) or mate defence (mammals) and hence related to mating strategies. Greenwood (1980) suggested that avian monogamy is consistent with a resource defence mating system which leads to female biased dispersal, whereas polygamy is linked to mammalian mate defence and male biased dispersal. In contrast to natal dispersal, our results imply that breeding dispersal will be dictated by the direction of polygamy, i.e. female biased in polyandrous population but male biased under polygyny. Two processes may explain why polygamous species have lower population divergence levels compared to monogamous species. In species with high sexual selection such as lekking species, males may either disperse to compete for additional mates, exploiting locally synchronised females (Kempenaers and Valcu 2017) or in the case of subordinate males they may disperse to find a space on a lek (Greenwood 1980). Habitat and mate availability may also be a strong factor driving female breeding dispersal in polyandrous species (Küpper *et al.* 2012, Cruz-Lopez *et al.* 2017).

In this study we are unable to determine the relative influence of natal versus breeding dispersal. To distinguish between the influence of natal and breeding dispersal on spatial genetic patterns, in addition to establishing whether dispersal patterns do truly differ between monogamous and polygamous species as predicted by the "dispersal-to-mate" hypothesis, further genetic, direct tracking and ringing studies are necessary. For example, a direct comparison of dispersal propensity between males and females within species representing different mating systems would provide strong evidence to support or refute the "dispersal-to-mate" hypothesis. Despite huge recent technological advances in direct tracking (Kays *et al.* 2015), methodological challenges such as the weight of tags have so far constrained our ability to compare detailed movement behaviour across an equivalent group of species as used in this study.

Contrary to our predictions, we found no support that annual migration influences spatial genetic patterns or subspecies richness in shorebirds. By undertaking seasonal

migration, one would predict that migratory species have a higher dispersal ability than resident species and that this may promote higher gene-flow between breeding populations (Winker, 2000; Claramunt *et al.* 2012, Weeks and Claramunt 2014). A possible reason for this is that migratory species may vary in their degree of migratory connectivity. Migratory connectivity is the strength of the association between a breeding site and a wintering site. Strong migratory connectivity is when individuals from one breeding ground always migrate to the same wintering ground, whereas, weak migratory connectivity reflects the mixing of populations on both breeding and wintering grounds (reviewed by Webster *et al.* 2002). Strong connectivity between breeding and wintering grounds can result in genetic divergence between populations (Rundel *et al.* 2013), however, the degree of connectivity is highly variable between and even within species (Rundel *et al.* 2013, Webster *et al.* 2002). Therefore, the presence or absence of genetic structure and variable IBD gradients within the six migrant plover species in our plover dataset as well as the variation in subspecies richness of migratory shorebirds, may reflect different levels of migratory connectivity between species. In addition, the migratory category of this study encompasses species which vary in different aspects of migration such as distance travelled, the proportion of the population migrating and wintering habitat, all of which could have implications for breeding site genetic structure and by proxy, subspecies richness. For example, Kraaijeveld (2008) found support for habitat stability affecting subspecies richness in shorebirds with species that overwinter at unstable inland wetlands showing lower subspecies numbers than those overwintering at coastal sites, which are characterized by more stable conditions. Habitat stability might also shape patterns of breeding dispersal with plovers breeding in volatile habitats being more likely to disperse than those breeding under stable conditions. Nevertheless, a higher propensity for dispersal might enable species to reach remote, isolated locations such as oceanic islands where they subsequently evolve into new species in allopatry (Phillimore *et al.* 2006). The exact use of species and subspecies delineation in avian taxonomy is currently debated, with disagreement about which species concept(s) are the easiest to operationalise (Sangster 2014, Barrowclough *et al.* 2016) and concerns about inappropriate grouping of populations (Gill 2014). We therefore decided to focus the plover analyses on continental populations only and because of the lack of similar genetic data for all shorebirds we did not evaluate subspecies delineation in the 136 shorebird species.

Present day spatial genetic patterns are the result of a multitude of past and present factors including demographic history (Excoffier 2004), habitat connectivity (Epps and Keyghobadi 2015) and range size (Phillimore *et al.* 2006). Although we did find that higher subspecies richness was associated with larger range sizes, supporting previous work (e.g. Salisbury *et al.* 2012), there was no such association within the plover data set. This is particularly interesting as two of the four polygamous species, Kentish and Kittlitz's plover, have extremely large breeding range sizes estimated at 13.6M km^2 and 16.4M km^2 (http://www.birdlife.org/datazone/species, accessed in: January 2017) respectively, yet both exhibit a distinct lack of continental genetic differentiation (see: Küpper *et al.* 2012 and dos Remedios, 2013), although their island populations are genetically differentiated.

Future studies are essential to further investigate the relationships between sexual selection, mate choice and breeding dispersal. New studies are needed to de-couple natal

and breeding sex-biased dispersal patterns and to compare these across species representing different mating systems. To test the broader relevance of the "dispersal-to-mate" hypothesis it is necessary to explore the theoretical basis of how selection for high mate access promotes dispersal and the population genetic consequences of this movement. Theoretical studies have been conducted to explain sex-biased dispersal in relation to mating systems (e.g. Kokko and Rankin 2006, Shaw and Kokko 2014), and these models provide excellent starting points for analysing mate access pressure, dispersal and gene-flow in relation to sexual selection.

In conclusion, we found that polygamous shorebirds exhibit reduced genetic differentiation compared to monogamous ones, consistently with a previous study carried out on Malagasy plovers (Eberhart-Phillips *et al.* 2015). These results oppose the notion that sexual selection promotes diversification per se. On the contrary, it appears that polygamy – usually associated with intense sexual selection – inhibits diversification in shorebirds by promoting gene-flow among distant continental sites. Future studies are needed to test the robustness of this hypothesis in other taxa with variation in mating systems.

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Table 2.1. Summary of sample characteristics for *Charadrius* plover species and populations included in genetic differentiation analyses. Mating system references are provided in Table S2.3. Information on breeding range size, mating system and migratory status are provided at species level. White-fronted and Kittlitz's plover mainland Africa populations were used only to corroborate spatial patterns found on Madagascar where sampling was more fine scale. Breeding range size is not provided for mainland Africa white-fronted and Kittlitz's plover populations. Loc Prior = different letters correspond to different location prior groupings for STRUCTURE analysis.

Species	Subspecies	Population (Map number | Loc Prior)	Latitude, longitude	N	Breeding range size (Km^2)	Mating system	Migratory status
piping plover	*circumcinctus*	Prairie North (1 | A)	53.2, -110.8	6			
Charadrius melodus	*circumcinctus*	Prairie South (2 | B)	51.4, -106.0	18			
Miller et al. 2010	*circumcinctus*	Great Plains North (3 | C)	47.6, -102.1	24			
	circumcinctus	Great Plains South (4 | D)	42.8, -97.4	23	221,000	Monogamous	Migratory
	circumcinctus	Great Lakes (5 | E)	45.8, -85.6	13			
	melodus	Atlantic Canada (6 | F)	45.9, -63.4	66			
	melodus	Atlantic USA (7 | G)	39.6, -73.8	70			
mountain plover	N/A	Northern (8 | A)	47.9, -107.9	21			
Charadrius montanus		Central (9 | B)	40.8, -104.0	34	759,000	Polygamous	Migratory
Oyler-McCance et al. 2008		Montane (10 | C)	39.3, -106.0	15			
		Southern (11 | D)	37.9, -103.1	24			
killdeer plover	*vociferus*	Summer Lake (12 | A)	42.8, -120.8	24			
Charadrius vociferous	*vociferus*	Honey Lake (13 | B)	40.3, -120.3	25	9,100,000	Monogamous	Migratory
(this study)	*vociferus*	Ceuta (14 | C)	23.9, -106.9	26			
snowy plover	*nivosus*	Utah (15 | A)	41.2, -112.3	25			
Charadrius nivosus	*nivosus*	San Quintín (16 | B)	30.6, -116.0	22			
(this study)	*nivosus*	Florida (17 | C)	29.9, -85.5	43	1,600,000	Polygamous	Migratory
	nivosus	Ceuta (18 | D)	23.9, -106.9	25			
	nivosus	Nayarit (19 | E)	22.4, -105.6	8			
	nivosus	Texcoco (20 | F)	19.5, -99.0	23			

Species	Subspecies	Population (Map number | Loc Prior)	Latitude, longitude	N	Breeding range size (Km^2)	Mating system	Migratory status
common ringed plover	*tundrae*	Lapland (21 | A)	68.4, 18.5	9			
Charadrius hiaticula	*tundrae*	Varanger (22 | B)	70.3, 30.7	12			
(this study)	*tundrae*	Northern Europe (23 | C)	67.7, 63.6	7			
	tundrae	Taimyr (24 | D)	72.9, 105.9	16			
	tundrae	North east Chukotka (25 | E)	67.1, -174.5	10	4,530,000	Monogamous	Migratory
	tundrae	East central Chukotka (26 | F)	64.7, 177.8	11			
	tundrae	South east Chukotka (27 | G)	62.5, 177.0	23			
	hiaticula	S.Sweden (28 | H)	57.3, 12.1	25			
	hiaticula	Belarus (29 | I)	52.1, 27.7	12			
Kentish plover	*alexandrinus*	Doñana (30 | A)	36.4, -6.4	25			
Charadrius alexandrinus	*alexandrinus*	Fuente de Piedra (31 | B)	37.1, -4.8	25			
Küpper et al., 2012	*alexandrinus*	Gharifa (32 | C)	35.2, -6.4	11			
	alexandrinus	Samouco (33 | D)	38.7, -8.9	25			
	alexandrinus	Beltringharder Koog (34 | E)	54.5, 8.9	13			
	alexandrinus	Kujalnik (35 | F)	46.8, 30.6	15	13,600,000	Polygamous	Migratory
	alexandrinus	Tuzla (36 | G)	36.7, 35.1	25			
	alexandrinus	Al Wathba (37 | H)	24.3, 54.6	25			
	alexandrinus	Lake Eton (38 | I)	49.1, 46.7	14			
	alexandrinus	Xinjiang (39 | J)	47.7, 87.5	7			
	alexandrinus	Bohai (40 | K)	39.1, 118.2	5			
Kittlitz's plover		Senegal 41 | Z)	16.4, -16.3	13			
Charadrius pecuarius		Gabon (42 | Y)	-0.5, 10.0	8			
dos Remedios, 2013	N/A	Kenya (43 | X)	-0.5, 36.3	28		Polygamous	Resident
African Mainland		Tanzania (44 | W)	-2.9, 35.9	2			
		Namibia (45 | V)	-18.9, 16.4	2			

Species	Subspecies	Population (Map number | Loc Prior)	Latitude, longitude	N	Breeding range size (Km^2)	Mating system	Migratory status
Kittlitz's plover		Namakia (54 | A)	-15.9, 45.8	29			
Charadrius pecuarius		Tsiribihina Delta (56 | B)	-19.7, 44.4	4			
Madagascar		Kirindy Mite (57 | C)	-20.9, 43.9	5			
Eberhart-Phillips et al., 2015		Fanjakana (58 | D)	-21.7, 45.1	3			
		Mangoky (59 | E)	-21.7, 43.4	2			
		Morombe (60 | E)	-21.8, 43.4	2	587,000	Polygamous	Resident
		Andavadoaka (61 | E)	-22.1, 43.3	28			
		Ifaty (62 | F)	-23.2, 43.6	2			
		Toliara Tsiongobory (63 | F)	-23.3, 43.6	2			
		Tsimanampetsotsa (66 | G)	-24.0, 43.7	30			
		Nosimborona (68 | G)	-25.1, 44.1	2			
Madagascar plover		Boanamary (51 | A)	-15.8, 46.3	2			
Charadrius thoracicus		Mahavavy (52 | A)	-15.8, 45.8	13			
Eberhart-Phillips et al., 2015		Marambitsy (53 | A)	-15.9, 45.7	17			
		Ankazobe (55 | B)	-17.3, 44.1	3			
		Kirindy Mite (57 | C)	-20.9 43.9	7			
	N/A	Mangoky (59 | C)	-21.7, 43.4	3	11,100	Monogamous	Resident
		Andavadoaka (61 | D)	-22.1, 43.3	24			
		Ifaty (62 | E)	-23.2, 43.6	4			
		Anakao (64 | F)	-23.7, 43.7	3			
		Besambay (65 | F)	-24.0, 43.7	5			
		Tsimanampetsotsa (66 | F)	-24.0, 43.7	28			
		Andranomasy (67 | F)	-24.2, 43.7	3			

Species	Subspecies	Population (Map number | Loc Prior)	Latitude, longitude	N	Breeding range size (Km^2)	Mating system	Migratory status
white-fronted plover	*marginatus*	Namibia (49 | Z)	-22.6, 14.5	18			
Charadrius marginatus	*marginatus*	South Africa (50 | Y)	-34.1, 18.4	11		Monogamous	Resident
dos Remedios 2013, African mainland							
white-fronted plover	*tenellus*	Marambitsy (53 | A)	-15.9, 45.7	39			
Charadrius marginatus	*tenellus*	Namikia (54 | A)	-15.9, 45.8	3			
Eberhart-Phillips et al., 2015	*tenellus*	Kirindy Mite (57 | B)	-20.7, 43.9	18	206,300	Monogamous	Resident
Madagascar	*tenellus*	Fanjakana (58 | C)	-21.7, 45.1	3			
	tenellus	Andavadoaka (61 | D)	-22.1, 43.3	32			
	tenellus	Tsimanampetsotsa (66 | E)	-24.1, 43.8	24			
chestnut banded plover	*venustus*	Kenya (46 | A)	-1.9, 36.3	12			
Charadrius pallidus	*venustus*	Tanzania (47 | A)	-2.9, 35.9	12	301,000	Monogamous	Resident
dos Remedios et al., 2017	*pallidus*	Namibia (48 | B)	-22.6, 14.5	39			

Chapter 2 Supplementary material:

Polygamy slows down population divergence in shorebirds

Data archival location: doi:10.5061/dryad.vn77k

PCR conditions for previously unpublished datasets.

Primers were arranged in multiplexes unique to each species. Total reaction volume was 10 µL which contained 5 µL mastermix (Qiagen, Valencia, California), ~2 µM of the primer mix, and 10 ng DNA. PCR started with a 15-min activation cycle at 95°C followed by 35 cycles of 94°C for 30 s, annealing temperature for 90 s (Table S1) and 90 s at 72°C, with a final extension for 10 min at 72°C.

Table S2.1. Microsatellite characteristics for all datasets. KD: Killdeer; KT: Kentish plover; SN: snowy plover; RG: common ringed plover; KZ-M: Kittlitz's plover (Madagascar); KZ-A: Kittlitz's plover (continental Africa); MD: Madagascar plover; WF-M: white fronted plover (Madagascar); WF-A: white fronted plover (continental Africa); MT: mountain plover; PP: piping plover; CB: chestnut banded. Shaded cell indicates high probability of null alleles in this locus. Null alleles were removed for STRUCTURE analysis and corrected for in all other analyses using FREENA software.

			Number of alleles											
Locus	**Reference**	**T_a °C**	**KD**	**KT**	**SN**	**RG**	**KZ-M**	**KZ-A**	**MD**	**WF-M**	**WF-A**	**MT**	**PP**	**CB**
BmaTATC371	Rew *et al.*, 2006	56	-	-	-	-	-	11	-	-	-	-	-	-
C201	Funk *et al.*, 2007	56	17	-	-	18	-	-	-	3	-	-	3	-
C204	Funk *et al.*, 2007	56	12	-	-	9	-	-	-	-	-	-	-	-
Calex01	Küpper *et al.*, 2007	56	-	12	2	-	7	8	4	-	3	-	-	-
Calex02	Küpper *et al.*, 2007	56	-	18	3	-	-	-	-	-	6	-	-	3
Calex04	Küpper *et al.*, 2007	56	-	11	2	-	-	-	-	-	3	-	-	4
Calex05	Küpper *et al.*, 2007	56	-	7	-	-	-	-	-	-	2	-	-	-
Calex06	Küpper *et al.*, 2007	56	-	-	-	-	3	-	2	11	-	-	-	-

Table S2.1. Continued

			Number of alleles											
Locus	**Reference**	**T_a °C**	**KD**	**KT**	**SN**	**RG**	**KZ-M**	**KZ-A**	**MD**	**WF-M**	**WF-A**	**MT**	**PP**	**CB**
Calex07	Küpper *et al.*, 2007	56	5	-	-	15	-	-	-	-	-	-	-	5
Calex08	Küpper *et al.*, 2007	56	3	7	-	-	-	-	-	-	2	-	4	6
Calex10	Küpper *et al.*, 2007	56	14	-	-	3	-	-	-	-	-	-	-	-
Calex11	Küpper *et al.*, 2007	56	-	11	1	-	-	-	-	-	3	-	-	-
Calex12	Küpper *et al.*, 2007	56	-	10	4	-	-	-	-	-	1	-	-	-
Calex13	Küpper *et al.*, 2008	56	-	-	-	-	-	-	-	-	-	-	2	-
Calex14	Küpper *et al.*, 2007	56	-	27	8	-	-	-	-	-	6	-	-	-
Calex15	Küpper *et al.*, 2007	56	-	-	-	-	-	4	-	-	-	-	-	-
Calex16	Küpper *et al.*, 2007	56	-	-	-	-	4	5	3	4	-	-	-	-
Calex17	Küpper *et al.*, 2007	56	6	-	-	4	-	-	-	-	-	-	-	-
Calex18	Küpper *et al.*, 2007	62	4	12	2	-	-	5	-	-	4	-	-	4
Calex19	Küpper *et al.*, 2007	56	-	15	2	-	7	6	6	3	3	-	-	4
Calex20	Küpper *et al.*, 2007	56	-	-	-	8	-	-	-	-	-	-	-	-
Calex22	Küpper *et al.*, 2007	56	3	7	-	-	-	-	-	-	4	-	-	-
Calex23	Küpper *et al.*, 2007	56	-	21	7	7	-	-	-	-	-	-	-	4
Calex24	Küpper *et al.*, 2007	56	-	7	2	-	-	-	-	-	-	-	-	-
Calex32	Küpper *et al.*, 2007	56	-	10	4	-	-	-	-	-	6	-	-	-
Calex33	Küpper *et al.*, 2007	56	-	-	-	-	10	11	6	-	-	-	-	5
Calex35	Küpper *et al.*, 2007	56	-	25	6	-	6	-	6	8	6	-	2	-
Calex36	Küpper *et al.*, 2007	62	-	-	-	-	7	-	3	2	-	-	-	-
Calex37	Küpper *et al.*, 2007	59	-	21	-	-	-	-	-	-	3	-	5	-
Calex39	Küpper *et al.*, 2007	56	-	31	5	-	-	-	-	-	5	-	-	5
Calex40	Küpper *et al.*, 2007	55-62	19	-	-	19	-	-	-	-	-	-	-	-
Calex43	Küpper *et al.*, 2007	56	4	25	4	-	-	-	2	-	6	-	-	-

Table S2.1. Continued

			Number of alleles											
Locus	**Reference**	**T_a °C**	**KD**	**KT**	**SN**	**RG**	**KZ-M**	**KZ-A**	**MD**	**WF-M**	**WF-A**	**MT**	**PP**	**CB**
Calex43b	Küpper *et al.*, 2008		-	-	-	-	2	-	-	9	-	-	-	-
GgaMan13	Piertney *et al.*, 2002	56	3	-	-	-	-	-	-	-	-	-	-	3
HrU2	Primmer *et al.*, 1995	56	-	7	6	-	-	-	-	-	4	-	-	-
Mopl2	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	3	-	-
Mopl5	St. John *et al.*, 2007	60	-	-	-	-	-	-	-	-	-	13	-	-
Mopl6	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	2	-	2
Mopl8	St. John *et al.*, 2007	63	-	-	-	-	-	-	-	-	-	5	-	-
Mopl9	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	7	-	-
Mopl13	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	4	-	-
Mopl15	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	3	-	-
Mopl17	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	4	-	-
Mopl18	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	2	-	-
Mopl19	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	2	-	-
Mopl21	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	4	-	-
Mopl22	St. John *et al.*, 2007	58	-	-	-	-	-	-	-	-	-	2	-	-
Mopl24	St. John *et al.*, 2007	55	-	-	-	-	-	-	-	-	-	13	-	-
Mopl26	St. John *et al.*, 2007	58	5	-	-	-	-	-	-	-	-	7	-	3
PLL10	Miller *et al.*, 2010	52	-	-	-	-	-	-	-	-	-	-	2	-
PLL11	Miller *et al.*, 2010	52	-	-	-	-	-	-	-	-	-	-	2	-
PLL4	Miller *et al.*, 2010	52	-	-	-	-	-	-	-	-	-	-	3	-
Tgu04_004	Dawson *et al.*, 2010	56	5	-	-	-	-	-	-	-	-	-	-	4
Tgu06	Slate *et al.*, 2007	56	10	-	-	-	-	7	-	-	-	-	-	2
Average number of alleles per locus			7.9	15.2	3.9	10.4	5.9	7.8	4.2	5.5	4.1	5.1	2.9	3.9
Total number of loci			14	20	15	8	9	9	9	8	18	14	8	15
Mantel test statistic			0.98	0.19	-0.1	0.24	-0.28	-	0.16	0.4	-	0.74	0.76	0.99
IBD gradient			5.42E-05	7.15E-07	-3.90E-06	3.47E-06	-6.71E-05	-	4.68E-05	4.37E-05	-	1.37E-05	6.57E-05	0.000208

Linear regression results testing for relationship between genetic diversity and the detection of spatial patterns

Average number of alleles ~ Mantel test statistic, estimate = -1.511, adjusted R^2 = -0.08, p = 0.62

Average number of alleles ~ isolation by distance gradient, estimate = -17131.63, adjusted R^2 = -0.005, p = 0.36

Number of loci ~ Mantel test statistic, estimate = 1.775, adjusted R^2 = 0.09, p = 0.62

Number of loci ~ isolation by distance gradient, estimate = 12344.81, adjusted R^2 = -0.079, p = 0.576

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Table S2.2. Migratory behaviour, mating system (MS; M: monogamous; P: polygamous), subspecies richness and breeding range size of 136 shorebird species. Migratory behaviour and breeding range size information was obtained from Birdlife International (http://www.birdlife.org/datazone/species, accessed in: July 2016). * = updated mating system information since Thomas et al., (2007). References (Ref) for mating are given in Table S2.3.

Genus	Species	Migratory	MS	N° subspp	Breeding range Km²	Log_{10} breeding range	Ref.
Actitis	*hypoleucos*	Migrant	M	1	25900000	7.413	50
Actitis	*macularius*	Migrant	P	1	13600000	7.134	34
Actophilornis	*africanus*	Resident	P	1	17000000	7.230	59
Anarhynchus	*frontalis*	Migrant	M	1	7700	3.886	58
Arenaria	*interpres*	Migrant	M	2	2620000	6.418	4
Arenaria	*melanocephala*	Migrant	M	1	83400	4.921	46
Bartramia	*longicauda*	Migrant	M*	1	3170000	6.501	16
Burhinus	*capensis*	Resident	M	4	12400000	7.093	26
Burhinus	*grallarius*	Resident	M	1	2570000	6.410	21
Burhinus	*oedicnemus*	Migrant	M	5	9150000	6.961	39
Burhinus	*senegalensis*	Resident	M	1	7040000	6.848	26
Burhinus	*vermiculatus*	Resident	M	2	5800000	6.763	52
Calidris	*acuminata*	Migrant	P	1	349000	5.543	17
Calidris	*alba*	Migrant	P*	2	1260000	6.100	5
Calidris	*alpina*	Migrant	M	10	4960000	6.695	31
Calidris	*bairdii*	Migrant	M	1	2810000	6.449	17
Calidris	*canutus*	Migrant	M	6	1600000	6.204	1
Calidris	*fuscicollis*	Migrant	P	1	419000	5.622	6
Calidris	*himantopus*	Migrant	M	1	399000	5.601	11
Calidris	*maritima*	Migrant	M	1	892000	5.950	69
Calidris	*mauri*	Migrant	M	1	310000	5.491	18
Calidris	*melanotos*	Migrant	P	1	2230000	6.348	7
Calidris	*minuta*	Migrant	P	1	1740000	6.241	48
Calidris	*minutilla*	Migrant	M	1	4970000	6.696	49
Calidris	*ptilocnemis*	Migrant	M	4	199000	5.299	51
Calidris	*pusilla*	Migrant	M	1	1220000	6.086	9
Calidris	*ruficollis*	Migrant	M	1	971000	5.987	73
Calidris	*temminckii*	Migrant	P	1	3450000	6.538	10
Calidris	*tenuirostris*	Migrant	M	1	1490000	6.173	73
Charadrius	*alexandrinus*	Migrant	P	4	13600000	7.134	55
Charadrius	*asiaticus*	Migrant	M	1	3030000	6.481	1
Charadrius	*bicinctus*	Migrant	M	2	580000	5.763	76
Charadrius	*dubius*	Migrant	M	3	19200000	7.283	1
Charadrius	*falklandicus*	Migrant	M*	1	809000	5.908	71
Charadrius	*forbesi*	Migrant	M	1	6930000	6.841	26
Charadrius	*hiaticula*	Migrant	M	3	4530000	6.656	74
Charadrius	*marginatus*	Resident	M	3	4440000	6.647	61
Charadrius	*melodus*	Migrant	M	2	221000	5.344	45
Charadrius	*modestus*	Migrant	M*	1	257000	5.410	57
Charadrius	*montanus*	Migrant	P	1	759000	5.880	33

Genus	Species	Migratory	MS	N° subspp	Breeding range Km²	Log₁₀ breeding range	Ref.
Charadrius	*morinellus*	Migrant	P	1	276000	5.441	68
Charadrius	*obscurus*	Migrant	M	2	310	2.491	77
Charadrius	*pallidus*	Resident	M	2	301000	5.479	26
Charadrius	*pecuarius*	Resident	P*	1	16300000	7.212	56
Charadrius	*peronii*	Resident	M*	1	1270000	6.104	60
Charadrius	*ruficapillus*	Resident	M*	1	4810000	6.682	53
Charadrius	*sanctaehelenae*	Resident	M*	1	46	1.663	78
Charadrius	*semipalmatus*	Migrant	M	1	1660000	6.220	75
Charadrius	*thoracicus*	Resident	M*	1	11100	4.045	61
Charadrius	*tricollaris*	Resident	M	2	9220000	6.965	26
Charadrius	*vociferus*	Migrant	M	3	9100000	6.959	13
Charadrius	*wilsonia*	Migrant	M*	4	843000	5.926	19
Thinornis	*novaeseelandiae*	Resident	M	2	3	0.477	79
Chionis	*albus*	Migrant	M	1	27100	4.433	80
Chionis	*minor*	Resident	M	4	8600	3.934	81
Coenocorypha	*aucklandica*	Resident	M	1	680	2.833	82
Coenocorypha	*pusilla*	Resident	M	1	5	0.699	82
Cursorius	*coromandelicus*	Resident	M*	1	2390000	6.378	62
Cursorius	*rufus*	Resident	M	1	1390000	6.143	26
Cursorius	*temminckii*	Migrant	M	3	10900000	7.037	26
Elseyornis	*melanops*	Resident	M	1	7350000	6.866	73
Erythrogonys	*cinctus*	Resident	M	1	5110000	6.708	43
Calidris	*pygmeus*	Migrant	M	1	61900	4.792	24
Gallinago	*gallinago*	Migrant	M	2	20100000	7.303	1
Gallinago	*media*	Migrant	P	1	6130000	6.787	1
Gallinago	*nigripennis*	Resident	M	3	497000	5.696	47
Glareola	*nordmanni*	Migrant	M	1	1440000	6.158	1
Glareola	*nuchalis*	Resident	M	2	6190000	6.792	26
Glareola	*pratincola*	Migrant	M	2	5350000	6.728	1
Haematopus	*bachmani*	Resident	M	1	593000	5.773	70
Haematopus	*fuliginosus*	Resident	M	2	812000	5.910	73
Haematopus	*leucopodus*	Resident	M*	1	228000	5.358	66
Haematopus	*longirostris*	Resident	M	1	855000	5.932	27
Haematopus	*moquini*	Resident	M	1	140000	5.146	38
Haematopus	*ostralegus*	Migrant	M	4	2780000	6.444	35
Haematopus	*palliatus*	Resident	M	2	863000	5.936	2
Haematopus	*unicolor*	Resident	M	1	73500	4.866	83
Himantopus	*himantopus*	Migrant	M	1	56700000	7.754	1
Hydrophasianus	*chirurgus*	Migrant	P	1	6410000	6.807	29
Irediparra	*gallinacea*	Resident	P	1	2180000	6.338	44
Jacana	*jacana*	Resident	P	6	13900000	7.143	30
Jacana	*spinosa*	Resident	P	1	1040000	6.017	3
Limicola	*falcinellus*	Migrant	M	2	1010000	6.004	1
Limnodromus	*griseus*	Migrant	M	3	1650000	6.217	2
Limosa	*fedoa*	Migrant	M	2	716000	5.855	42
Limosa	*lapponica*	Migrant	M	4	1470000	6.167	1
Limosa	*limosa*	Migrant	M	3	7180000	6.856	1
Metopidius	*indicus*	Resident	P	1	2650000	6.423	8

Genus	Species	Migratory	MS	N° subspp	Breeding range Km²	Log10 breeding range	Ref.
Microparra	*capensis*	Resident	M	1	3240000	6.511	23
Numenius	*americanus*	Migrant	M	1	1820000	6.260	49
Numenius	*arquata*	Migrant	M	3	6800000	6.833	1
Numenius	*phaeopus*	Migrant	M	7	4790000	6.680	1
Numenius	*tahitiensis*	Migrant	M	1	45300	4.656	22
Pedionomus	*torquatus*	Resident	P	1	32600	4.513	40
Phalaropus	*fulicarius*	Migrant	P	1	3900000	6.591	49
Phalaropus	*lobatus*	Migrant	P	1	5110000	6.708	65
Phalaropus	*tricolor*	Migrant	P	1	3820000	6.582	20
Philomachus	*pugnax*	Migrant	P	1	8580000	6.933	1
Pluvialis	*apricaria*	Migrant	M	1	1170000	6.068	1
Pluvialis	*dominica*	Migrant	M	1	1440000	6.158	32
Pluvialis	*fulva*	Migrant	M	1	1730000	6.238	49
Pluvialis	*squatarola*	Migrant	M	3	3980000	6.600	1
Pluvianus	*aegyptius*	Resident	M	1	5980000	6.777	1
Recurvirostra	*americana*	Migrant	M	1	1390000	6.143	25
Recurvirostra	*avosetta*	Migrant	M	1	12800000	7.107	36
Rhinoptilus	*africanus*	Resident	M	8	3000000	6.477	63
Rostratula	*benghalensis*	Resident	P	1	23400000	7.369	1
Scolopax	*minor*	Migrant	P	1	1600000	6.204	15
Scolopax	*rusticola*	Migrant	P	1	15100000	7.179	84
Tringa	*erythropus*	Migrant	M	1	3720000	6.571	1
Tringa	*flavipes*	Migrant	M	1	4590000	6.662	49
Tringa	*glareola*	Migrant	M	1	15500000	7.190	1
Tringa	*nebularia*	Migrant	M	1	12100000	7.083	72
Tringa	*ochropus*	Migrant	M	1	14900000	7.173	1
Tringa	*solitaria*	Migrant	M	2	5550000	6.744	49
Tringa	*stagnatilis*	Migrant	M	1	6300000	6.799	1
Tringa	*totanus*	Migrant	M	6	19700000	7.294	1
Tryngites	*subruficollis*	Migrant	P	1	599000	5.777	12
Vanellus	*albiceps*	Resident	M	1	7540000	6.877	26
Vanellus	*armatus*	Resident	M	1	5660000	6.753	26
Vanellus	*chilensis*	Resident	M*	4	13200000	7.121	37
Vanellus	*cinereus*	Migrant	M*	1	549000	5.740	67
Vanellus	*coronatus*	Resident	M	3	6790000	6.832	26
Vanellus	*crassirostris*	Resident	M	2	3460000	6.539	26
Vanellus	*gregarius*	Migrant	M	1	1500000	6.176	1
Vanellus	*indicus*	Resident	M	4	5220000	6.718	1
Vanellus	*lugubris*	Resident	M	1	3560000	6.551	26
Vanellus	*melanocephalus*	Resident	M	1	180000	5.255	26
Vanellus	*melanopterus*	Resident	M	2	839000	5.924	26
Vanellus	*miles*	Resident	M	2	3050000	6.484	14
Vanellus	*senegallus*	Resident	M	2	11300000	7.053	26
Vanellus	*spinosus*	Resident	M	1	8170000	6.912	1
Vanellus	*superciliosus*	Migrant	M	1	752000	5.876	26
Vanellus	*tectus*	Resident	M	2	5270000	6.722	26
Vanellus	*tricolor*	Resident	M	1	5640000	6.751	43
Vanellus	*vanellus*	Migrant	P*	1	6700000	6.826	54

Table S2.3. References for mating system information of 136 shorebird species used in PGLS analysis.

Ref.	Author	Year	Title	Journal/Publisher	Vol	Pages / DOI/ web link
1	Cramp, S. and Simmons, K.E.L.	1983	The Birds of the Western Palearctic Vol 3	OUP, Oxford		
2	Johnsgard, P.A.	1981	The Plovers, Sandpipers, and Snipes of the World	University of Nebraska Press, Lincoln and London		
3	Jenni, D.A. and Collier, G.	1972	Polyandry in the American Jacana (*Jacana spinosa*)	Auk	89	743-765
4	Nettleship, D.N.	1973	Breeding ecology of turnstone Arenaria interpres at Hazen Camp, Ellesmere Island, NWT	Ibis	115	202-217
5	Reneerkens, J., van Veelen, P., van der Velde, M., Luttikhuizen, P., and Piersma, T.	2014	Within-population variation in mating system and parental care patterns in the Sanderling (*Calidris alba*) in northeast Greenland	Auk	131	235-247
6	Parmelee, D.F., Greiner, D.W. and Graul, W.D.	1968	Summer schedule and breeding biology of the white-rumped sandpiper in the Central Canadian Arctic	Wilson Bulletin	80	5-29
7	Pitelka, F.A.	1959	Numbers, breeding schedule, and territoriality in pectoral sandpipers of northern Alaska	Condor	61	233-264
8	Art, S.H.B.	2000	Population structure and breeding system of the sex-role reversed, polyandrous Bronze-winged Jacana *Metopidius indicus*	Ibis	142	93-102
9	Gratto-Trevor, C.	1991	Parental care in Semipalmated Sandpipers *Calidris pusilla*: brood desertion by females	Ibis	133	394-399
10	Hilden, O.	1975	Breeding system of Temminck's Stint *Calidris temminckii*	Ornis Fennica	52	117-146
11	Jehl, J.R., Jr.	1973	Breeding biology and systematic relationships of the stilt sandpiper	Wilson Bulletin	85	115-147
12	Pitelka, F.A., Holmes, R.T. and S.F. MacLean, Jr.	1974	Ecology and Evolution of Social Organization in Arctic Sandpipers	American Zoologist	14	185-204

13	Lenington, S.	1980	Bi-parental care in killdeer: an adaptive hypothesis	Wilson Bulletin	92	8-20
14	Cardilini, A. P., Weston, M. A., Dann, P., and Sherman, C. D.	2015	Sharing the Load: Role Equity in the Incubation of a Monomorphic Wader, the Masked Lapwing (*Vanellus miles*)	Wilson Journal of Ornithology	127	730-733
15	Mendall, H.L. and Aldous, C.M.	1943	The ecology and management of the American Woodcock	Maine Cooperative Wildlife Research Unit, Orne, Maine		
16	Casey, A. E., Sandercock, B. K., & Wisely, S. M.	2011	Genetic parentage and local population structure in the socially monogamous upland sandpiper	Condor	113	119-128
17	Myers, J.P., Hildén, O. and Tomkovich, P.	1982	Exotic Calidris species of the Siberian tundra	Ornis Fennica	59	175-182
18	Holmes, R.T.	1973	Social behaviour of breeding western sandpipers *Calidris mauri*	Ibis	115	107-123
19	Cox., L. M.	2015	Breeding Biology of Wilson's Plovers (*Charadrius Wilsonia*): Reproductive Success, Habitat Use, and Sex Roles	Georgia southern University, MSc thesis	1269	http://digitalcommons.georgiasouthern.edu/etd/1269
20	Delechanty, D. J., Fleischer, R.C., Colwell, M. A. and Oring, L.W	1998	Sex-role reversal and the absence of extra-pair fertilization in Wilson's phalaropes	Animal Behaviour	55	995-1002
21	Anderson, G.J.	1991	The breeding biology of the bush thick-knee *Burhinus magnirostris* and notes on its distribution in the Brisbane area	Sunbird	21	33-61
22	Gill, R.E., Lanctot, R.B., Mason, J.D. and Handel, C.M.	1991	Observations on habitat use, breeding chronology and parental care in Bristle-thighed Curlews on the Seward Peninsula, Alaska	Wader Study Group Bulletin	61	28-36
23	Tarboton, W.R. and Fry, C.H.	1986	Breeding and other behaviour of the lesser jacana	Ostrich	57	233-243
24	Tomkovich, P.S.	1995	Breeding biology and breeding success of the spoon-billed sandpiper *Eurynorhynchus pygeus*	Russian Journal of Ornithology. In Russian with English Summary	85	29-34

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26	Urban, E.K., Fry, C.H. and Keith, S.	1986	The Birds of Africa Vol II	Academic Press, London		
27	Wakefield, W.C.	1988	Breeding resource partitioning of a mixed population of pied and sooty oystercatchers	Stilt	13	39-40
28	Barlow, M.L., Muller, P.M. and Sutton, R.R.	1972	Breeding data on the spur-winged plover in southland, New Zealand	Notornis	19	212-249
29	Pringle, J.D.	1987	The Waders of Australia	Angus and Robertson Publishers, North Ryde		
30	Osborne, D.R.	1982	Replacement nesting and polyandry in the Wattled Jacana	Wilson Bulletin	94	206-208
31	Flodin, L. Å., and Blomqvist, D.	2012	Divorce and breeding dispersal in the dunlin Calidris alpina: support for the better option hypothesis?	Behaviour	149	67-80
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34	Oring, L.W. and Lank, D.B.	1984	Breeding area fidelity, natal philopatry, and the social systems of sandpipers In: Eds Burger, J and Olla, BL Waders Breeding Behavior and Populations Behavior of Marine Animals Vol. 5	Plenum Press, New York		125-147
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41	Höhn, E.O.	1975	Notes on black-headed ducks, painted snipe, and spotted tinamous	Auk	92	566-575
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43	Marchant, S. and P.J. Higgins	1993	Handbook of Australian, New Zealand and Antarctic birds Vol 2	OUP, Oxford		
44	Jenni, D.A.	1996	Family Jacanidae (Jacanas) In: J del Hoyo, A Elliott and J Sargatal (eds), Handbook of the Birds of the World (Vol. 3 - Hoatzin to Auks)	Lynx Editions , Spain		
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60	Yasué, M., and Dearden, P.	2008	Replacement nesting and double-brooding in Malaysian Plovers *Charadrius peronii*: effects of season and food availability	Ardea	96	59-72
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65	Schamel, D., Tracy, D. M., Lank, D. B., and Westneat, D. F.	2004	Mate guarding, copulation strategies and paternity in the sex-role reversed, socially polyandrous red-necked phalarope *Phalaropus lobatus*	Behavioral Ecology and Sociobiology,	57	110-118
66	Newman, M.	2013	Brood capture by Australian pied oystercatchers	Stilt	63 - 64	16-21
67	Takahashi, M., and Ohkawara, K	2007	Breeding behavior and reproductive success of Grey-headed Lapwing *Vanellus cinereus* on farmland in central Japan	Ornithological science	6	1-9
68	Owens, I. P., Dixon, A., Burke, T., and Thompson, D. B.	1995	Strategic paternity assurance in the sex-role reversed Eurasian dotterel (*Charadrius morinellus*): behavioral and genetic evidence	Behavioral Ecology	6	14-21
69	Pierce, E. P., and Lifjeld, J. T.	1998	High paternity without paternity-assurance behavior in the Purple Sandpiper, a species with high paternal investment	Auk		602-612
70	Spiegel, C. S. (2008).	2008	Incubation patterns, parental roles, and nest survival of black oystercatchers (*Haematopus bachmani*): influences of environmental processes and potential disturbance stimuli	Oregon state university PhD thesis		http://hdl.handle.net/1957/10239
71	St Clair, J. J., Herrmann, P., Woods, R. W., and Székely, T.	2010	Female-biased incubation and strong diel sex-roles in the Two-banded Plover *Charadrius falklandicus*	Journal of Ornithology	151	811-816
72	Thompson, P. S., and Thompson, D. B. A.	1991	Greenshanks *Tringa nebularia* and long-term studies of breeding waders	Ibis	133	99-112
73	Tomkovich, P. S., and Weston, M. A.	2007	Breeding Ecology IN: 'Shorebirds of Australia'(Eds A Geering, L Agnew and S Harding)	CSIRO Publishing, Clayton		

74	Wallander, J, Blomqvist, D. and Lifjeld, J.T.	2001	Genetic and social monogamy–does it occur without mate guarding in the common ringed plover?	Ethology	107	561-572
75	Zharikov, Y., and Nol, E.	2000	Copulation behavior, mate guarding, and paternity in the Semipalmated Plover	Condor	102	231-235
76	Pierce, R. J.	1989	Breeding and social patterns of banded dotterels (*Charadrius bicinctus*) at Cass River	Notornis	36	13-23
77	Dowding, J. E., Wills, D. E., & Booth, A. M.	1999	Double-brooding and brood overlap by Northern New Zealand Dotterels (*Charadrius obscurus aquilonius*)	Notornis	46	181-186
78	Burns, F., McCulloch, N., dos Remedios, N., Bolton, M., & Szekely, T.	2013	Sex differences in incubation behaviour but not mortality risk in a threatened wader	Ibis	155	877-880
79	Davis, A.	1994	Breeding biology of the New Zealand shore plover *Thinornis novaeseelandiae*	Notornis	41	195-208
80	Parmelee, D. F.	1993	Antarctic birds: ecological and behavioural approaches	Antarctic Science	5	227-232
81	Burger, A. E.	1979	Breeding biology, moult and survival of Lesser Sheathbills *Chionis minor* at Marion Island	Ardea	67	1-14
82	Miskelly, C. N.	1990	Breeding systems of New Zealand Snipe *Coenocorypha aucklandica* and Chatham Island Snipe *C. pusilla*; are they food limited?	Ibis	132	366-379
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84	Hirons, G.	1983	A five-year study of the breeding behaviour and biology of the woodcock in England - A first report	Proc. 2nd European Woodcock and Snipe Workshop		51-67

Table S2.4. Single parameter phylogenetic least squares analysis (General linear model) model selection testing the association between isolation by distance gradient of *Charadrius* plovers and 1) mating system, 2) migratory status, and 3) breeding range size, df = degrees of freedom, AICc = Akaike information criterion corrected for small sample size.

Model	df	Intercept	delta	t-value	p-value	weight	AICc
1	**8**	**4.19E-05**	**0.00**	**-2.49**	**0.05**	**0.86**	**-138.9**
(mating system)		**(7.03E-05)**	**(0.00)**	**(-2.18)**	**(0.06)**	**(0.78)**	**(-158.3)**
2	8	2.24E-05	4.46	-0.99	0.36	0.09	-134.4
(migratory or resident)		(1.59E-04)	(3.90)	(0.75)	(0.48)	(0.11)	(-154.4)
3	8	6.65E-05	5.54	-0.36	0.73	0.05	-133.3
(log breeding range size)		(2.24E-05)	(3.98)	(-0.79)	(0.45)	(0.11)	(-154.3)

Table S2.5 Phylogenetic least squares analysis model simplification results to test explanatory variables (mating system, migratory status and breeding range size) on the subspecies richness of 136 shorebird species. df = degrees of freedom. AIC = Akaike information criterion.

Model	Effect	estimate		*t*- value	*p*-value	AIC	df
1	Mating system	-0.890	± 0.384	-2.315	0.022		
1	Migratory status	-0.144	± 0.295	-0.487	0.627		131
1	Mating system : Migratory status	0.545	± 0.692	0.787	0.433	500.23	
1	Log10 breeding range size	0.211	± 0.113	1.861	0.065		
2	Mating system	-0.724	± 0.321	-2.255	0.026		
2	Migratory status	-0.044	± 0.266	-0.165	0.869	498.87	132
2	Log10 breeding range size	0.218	± 0.113	1.935	0.055		
3	**Mating system**	-0.719	± 0.318	-2.258	**0.026**	496.90	133
3	**Log10 breeding range size**	0.221	± 0.111	1.979	**0.050**		

Table S2.6 Pairwise F_{ST} between subspecies of (A) ringed plover (*Charadrius hiaticula*), (B) chestnut banded plover (*Charadrius pallidus*) and (C) white-fronted plover (*Charadrius marginatus*). Significance at p<0.001is indicated by *.

(A)

	hiaticula	*tundrae*
hiaticula	0	
tundrae	0.0109*	0

(B)

	pallidus	*venustus*
pallidus	0	
venustus	0.4157*	0

(C)

	marginatus	*tenellus*
marginatus	0	
tenellus	0.5648*	0

Post Chapter 2 Commentary

In Chapter 2, I found support for the prediction that polygamous species are characterised by weak population structure and fewer subspecies compared to monogamous species. I interpreted this pattern as a consequence of higher dispersal in polygamous species compared to monogamous species, driven by pressure to find multiple breeding partners. I found no support, however, for the association between high dispersal capacity and population divergence. The evidence that sexual selection pressure may restrict the divergence of populations is a stark contrast to its traditional description as an "engine of speciation".

The only outlier species from cluster analysis was the snowy plover. Interestingly this is a polyandrous species (Warriner *et al.* 1986, Page et al. 2009, Eberhart-Phillips *et al.* 2017) with documented long distance breeding dispersal of females (Stenzel *et al.* 1994). Findings from cluster analysis indicate sub-structuring within the *Charadrius nivosus nivosus* subspecies, however, the significance of this is unclear with limited genetic data.

Importantly, what I describe in this chapter is an association rather than causation, therefore, further investigations into the mechanisms behind this relationship are required. Specifically, this hypothesis is based under the assumption that the polygamous sex drives the gene-flow in polygamous mating systems. I was unable to test this assumption using the microsatellite datasets available because they were all autosomal, and the populations within each species were sampled at different time points (but see Banks and Peakall 2012, Prugnolle and Meeûs 2002).

There are two main lines of future enquiry from this Chapter. Firstly, to examine the evolutionary history and gene-flow of snowy plover populations using a more comprehensive genetic dataset. Secondly, to determine whether gene-flow is biased toward the promiscuous sex in polygamous species.

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Appendix B: Statement of Authorship

<table>
<tr><th colspan="10">This declaration concerns the article entitled:</th></tr>
<tr><td colspan="10">Population differentiation and demography of the threatened snowy plover Charadrius nivosus estimated by four different genetic markers.</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td>X</td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td></td></tr>
<tr><td>Publication details (reference)</td><td colspan="9"></td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">J.D.J conducted bioinformatics processing for ddRAD data with advice from C.K., J.D.C and M.D.S. J.D.J. designed methodology with C.K. J.D.J executed all population genetic data analyses with advice from co-authors. J.D.J wrote the first draft of the manuscript. The manuscript has been improved further by co-authors. All figures were created by J.D.J. J.D.J. contribution 80%</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
<tr><td>Signed</td><td colspan="6"></td><td>Date</td><td colspan="2"></td></tr>
</table>

Commentary Pre Chapter 3

In my third Chapter, I examine the evolutionary history, subspecies delineations, and sex-biased gene-flow of snowy plover populations. I achieve this by combining all available snowy plover genetic resources, in addition to analysing newly acquired mitochondrial DNA (mtDNA) sequences, microsatellite genotypes and double digest restriction enzyme site associated DNA (ddRAD) sequences. With these data I compare the differentiation and diversity of bi-parentally (autosomal), paternally (Z chromosome), and maternally (mtDNA) inherited markers to examine if, consistent with the "dispersal-to-mate" hypothesis from Chapter 2, gene-flow is female biased in this polyandrous species. In female heterogametic taxa such as birds, the Z chromosome spends twice as much evolutionary time in males compared to females, therefore, genetic patterns of this genomic region largely reflect the male germline (Ellegren 2009). Whereas, mtDNA is largely maternally inherited and shows female evolutionary history (but see Barr *et al.* 2005). In addition, mtDNA is often used to identify conservation units (Moritz 1994). I also combine mtDNA and nuclear data to determine if the genetic cluster representing Florida in Chapter 2 is supported by multiple genetic markers, and examine the demographic history of snowy plover genetic clusters.

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3

Population differentiation and demography of the threatened snowy plover *Charadrius nivosus* estimated by four different genetic markers

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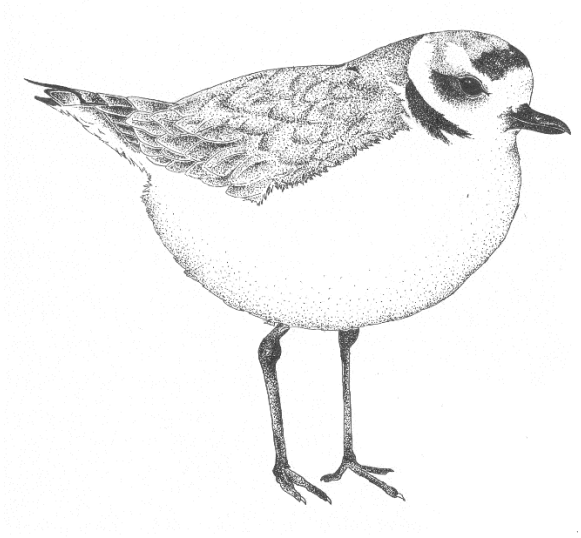

Snowy plover by Becca Young

Author contributions

J.DJ. Conducted analyses; manuscript preparation
M.W.B and T.S. Advice on methodologies; manuscript improvement
J.D. and M.S. ddRAD library preparation; advice on methodologies; manuscript improvement
S.V.E Idea conception, grant funding
I.-R.R. Advice on methodologies; manuscript improvement
K.M. Microsatellite genotyping
M.C.-L.; D.G-E.; A.E.D.S-M; J.C; R.P.; A.L.M and O.G. sample collection
T.B. Microsatellite and mitochondrial sequencing facilities; funding
C.K. Idea conception; grant funding; advice on methodologies, manuscript improvement.

Population differentiation and demography of the threatened snowy plover *Charadrius nivosus* estimated by four different genetic markers

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Abstract

Delineating conservation units is a complex and often controversial process that is particularly challenging for highly dispersive species. Here, we use newly available genetic data to reassess genetic population structure and identify populations for conservation prioritisation in the threatened snowy plover (*Charadrius nivosus*), a partial migrant shorebird endemic to the Americas. Our investigation combines four genetic markers: mitochondrial (mt) DNA, microsatellites, Z-linked and autosomal single nucleotide polymorphisms (SNPs), to 1) assess current subspecies delineation and examine population structure within the most widespread subspecies, *C. n. nivosus*, 2) compare the power of the markers used to detect fine scale genetic structure and isolation by distance among contested population segments, and 3) reconstruct the demographic history of snowy plover populations. Current subspecies delineations were broadly supported by all markers, although, microsatellite and SNPs, but not mtDNA provided support for Floridian snowy plovers constituting a distinct population segment (eastern snowy plovers). We found low genetic exchange between subspecies, as reflected by low migration rates using autosomal SNPs (0.02-0.04), but significant unidirectional migration (0.30) from western *nivosus* populations into the eastern *nivosus* segment. In contrast, we did not detect genetic differentiation between coastal Pacific and inland populations. Significant isolation by distance was detected with every marker when including all three subspecies, and also for three of the four markers within the *nivosus* subspecies alone. All populations showed low genetic diversity and signatures of strong bottlenecks occurring during the last 1000 years. We suggest that

conservation management and monitoring is prioritised on the *tenuirostris* and *occidentalis* subspecies. Future studies should combine genomic, demographic and movement data in a holistic approach to determine snowy plover population connectivity.

Keywords: *Charadrius nivosus*, snowy plover, microsatellite, ddRAD, mtDNA, demography, subspecies

Introduction

Distributing conservation efforts below the species level requires the prioritisation of specific populations and defining appropriate conservation units. This is often a highly political (Haig *et al.* 2016, Cook and Sgrò 2017), challenging, and complex process that involves the consideration of multiple factors. These factors include, how "unique" the population is (Waples 1991, Moritz 1994), the level of genetic diversity it harbours (Hoban *et al.* 2013a), recent and/or projected demographic changes, and population connectivity (Mills and Allendorf 1996, Waples and Gaggiotti 2006, Lankau and Strauss 2011, Lowe *et al.* 2017).

The delineation of conservation units such as evolutionary significant units (ESUs, Moritz 1994) and management units (MUs) is highly nuanced and can be difficult to define in practice because of species specific ecology and life history traits. For example, highly dispersive taxa such as pelagic organisms and species capable of flight are unlikely to have many reciprocally monophyletic groups based on their mitochondrial (mtDNA) sequence, simply due to high rates of gene-flow (Crandall *et al.* 2000, Medina *et al.* 2018). Traditionally, conservation units have been identified using a small number of variable loci such as mtDNA and microsatellite markers. However, a greater number of genetic markers increases the power to detect fine scale structure, and hence, increases the reliability of defining conservation units and their associated characteristics such as past demographic changes and effective population sizes (Allendorf *et al.* 2010, Funk *et al.* 2012, Shafer *et al.* 2015a). Genomic methods, including restriction enzyme associated DNA sequencing (RADseq, Baird *et al.* 2007, Miller *et al.* 2007), produce hundreds or thousands rather than tens of markers distributed throughout the genome, and can reveal previously uncharacterised genetic structure (e.g. Ruegg *et al.* 2014, Saenz-Agudelo *et al.* 2015, Barth *et al.* 2017, Vendrami *et al.* 2017, Younger *et al.* 2017). In doing so, genomic methods have enabled novel, high resolution identification of management units in species of high conservation priority (Palsbøll *et al.* 2007, Kjeldsen *et al.* 2016, Peters *et al.* 2016, Barth *et al.* 2017, Younger *et al.* 2017). Although increased genomic sampling does not guarantee the detection of cryptic population structure, it has proven successful in corroborating previously proposed taxonomic or conservation units that were designated using low resolution genetic data (e.g. Mason and Taylor 2015, Attard *et al.* 2018, Doyle *et al.* 2018) or ecological variation (e.g. Lemay and Russello 2015, Prince *et al.*, 2017).

Snowy plovers, *Charadrius nivosus,* are one of the least abundant shorebirds endemic to the Americas (Küpper *et al.* 2009) but one of the best studied. This iconic, partially migratory shorebird exhibits the unusual breeding system of sequential polyandry, and inhabits salt flats of alkaline lakes, coastal lagoons and sandy beaches (Warriner *et al.* 1986, Page *et al.* 2009, Eberhart-Phillips *et al.* 2017). Three subspecies are currently recognised (Figure 3.1, American Ornithologists' Union 1957, Funk *et al.* 2007).

Subspecies *nivosus* is found from the Pacific United States coast to Lousiana, *tenuirostris* inhabits the Caribbean Islands and the Eastern Gulf of Mexico, whereas, subspecies *occidentalis* is found along the west coast of South America from Colombia to Chile. The estimated census population sizes of the three subspecies are 25,869 (*nivosus,* Thomas *et al.* 2012), 8,000-10,000 (*occidentalis,* Wetlands International 2014) and 2,500 (*teniurostris*, Wetlands International 2014). Coastal populations are declining because of habitat deterioration and disturbance (Colwell *et al.* 2007, Küpper *et al.* 2011, Powell and Collier 2011, Page *et al.* 2009, Thomas *et al.* 2012, Cohen *et al.* 2014, Galindo-Espinosa and Palacios 2015, Cruz-López *et al.* 2017). As a result, many populations are protected by either federal or state-wide legislation (Haig *et al.* 2011, Cohen *et al.* 2014, Galindo-Espinosa and Palacios 2015). Population viability models suggest that the decline of coastal populations will become more severe throughout their range without intensive conservation management (Aiello-Lemmins *et al.* 2011, Eberhart-Phillips and Colwell 2014, Cruz-Lopéz *et al.* 2017). Furthermore, climate change might exacerbate risks of local extinctions due to rising sea levels and changes in the dynamics of tropical cyclone events, which are important for creating suitable nesting habitats (Aiello-Lemmins *et al.* 2011, Convertino *et al.* 2011).

A previous genetic analysis of snowy plovers found support for the existing three subspecies, however, the authors suggested changes in the delineation among them (Funk *et al.* 2007). Specifically, snowy plovers breeding in Florida and at the Gulf of Mexico (eastern snowy plover) that had been previously assigned to subspecies *tenuirostris* were more genetically similar to those of the subspecies *nivosus* (Funk *et al.* 2007). However, a more recent analysis based on microsatellite markers with additional sampling of Mexican populations showed that snowy plovers from Florida may be differentiated from the *nivosus* snowy plovers (D'Urban Jackson *et al.* 2017). Funk *et al.* (2007) and D'Urban Jackson *et al.* (2017) failed to find genetic support for separate conservation management of Pacific coastal populations. Despite this, the conservation status of Pacific snowy plovers as a distinct population segment under the Endangered Species Act was maintained, because of the lack of banding data available to confirm movement between inland and coastal sites (Haig *et al.* 2011). High levels of gene-flow between western *nivosus* populations could be the result of long distance breeding dispersal of females (D'Urban Jackson *et al.* 2017) which has been reported from Pacific populations (Stenzel *et al.* 1994). Previous studies that used microsatellites and mtDNA sequences have described low genetic diversity across snowy plover subspecies (Funk *et al.* 2007, D'Urban Jackson *et al.* 2017). Furthermore, inbreeding has been detected in a small isolated population of snowy plovers through a long-term pedigree study (Colwell and Pearson 2011).

Both previous genetic analyses (Funk *et al.* 2007, D'Urban Jackson *et al.* 2017) used ≤15 loci, which may have limited the detection of fine scale population structure. Here, we comprehensively re-evaluate the delineation of snowy plover populations using four different genetic markers: mtDNA, microsatellites, autosomal and Z-linked single-nucleotide polymorphisms (SNPs). Snowy plover breeding habitat includes sandy beaches, therefore, the conservation management of this species in North America remains controversial, as habitat protection often conflicts with development and human recreational activities (Lafferty *et al.* 2006). Regular reassessment of population structure in snowy plovers implementing methodological advances is, therefore, required to guide an effective conservation policy. We 1) assess current subspecies delineation and examine population structure within the most widespread subspecies *nivosus*, 2) compare the power of the four genetic markers to detect fine scale genetic

structure among controversial population segments, 3) test for isolation by distance within and across subspecies and 4) reconstruct the demographic history of snowy plover populations to understand the origin of low genetic diversity and suggest conservation priorities.

Materials and methods

We gathered genetic data from over 410 samples comprising all three *C. nivosus* subspecies using previously published (Funk *et al.* 2007, Küpper *et al.* 2009, D'Urban Jackson *et al.* 2017) and newly collected data. Sampling sites for each marker are shown in Figure 3.1 and sample information is summarised in Table 3.1, with a more detailed description for each marker type below.

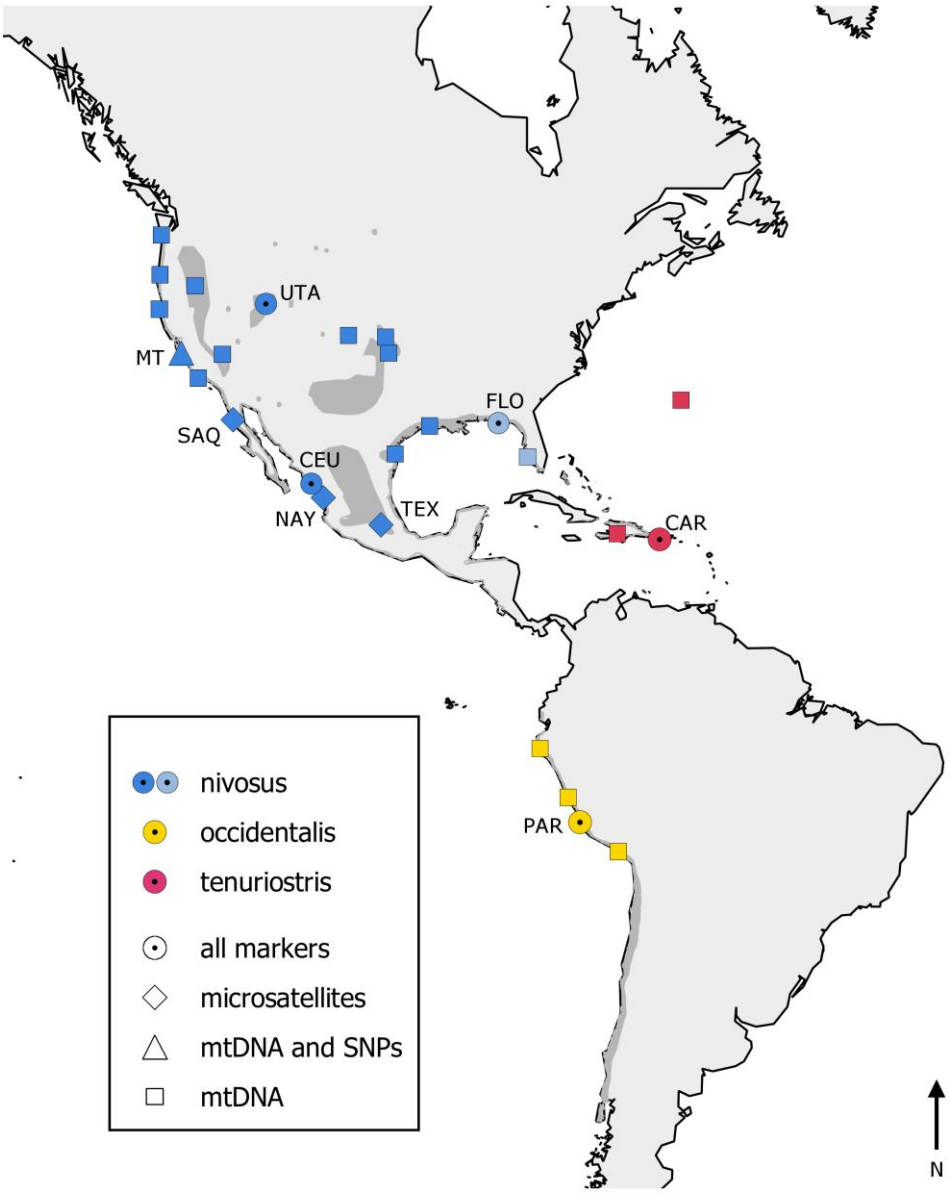

Figure 3.1. Sampling locations of snowy plovers (*Charadrius nivosus*) for four genetic markers (see legend). Genetic deme identity based on nuclear autosomal markers is given by colour (blue: western *nivosus*, light blue: eastern *nivosus*, red: *tenuirostris*, yellow: *occidentalis*). Species range is indicated in dark grey shading. For details on sample sizes see Table 3.1 (all markers) and Table S3.6 (mtDNA only).

Microsatellites

Samples from subspecies *occidentalis* (n=18) and *tenuirostris* (n=13) were added to those described in the microsatellite dataset for *nivosus* published in D'Urban Jackson *et al.* (2017). We genotyped all samples at 15 microsatellite loci (including seven additional markers in comparison to D'Urban Jackson *et al.* (2017)) and removed first order relatives based on field records. Primers, PCR conditions and reagents can be found in Küpper *et al.* (2008, 2009) and D'Urban Jackson *et al.* (2017).

mtDNA

We downloaded available snowy plover sequences of the mitochondrial control region (D-loop) from Genbank (Table S3.6). In addition, we sequenced the D-loop for 67 extra samples using primers and PCR conditions described in Küpper *et al.* (2012). We aligned all sequences using the ClustalW alignment tool in BioEdit v7.2.5 (Hall 1999) and trimmed them to 424 bp for downstream analysis. Locations with only mtDNA data are described in Table S3.6 and in Funk *et al.* (2007). For locations with additional genetic information (microsatellites or SNPs) see Table 3.1 and Figure 3.1.

SNPs

We extracted DNA from blood samples using the phenol-chloroform extraction method (Sambrook and Russell 2006). We prepared a multiplexed (n=47 individuals) library for double digest restriction site associated DNA sequencing (ddRAD) following the protocol described by DaCosta and Sorenson (2014) using *EcoR*I and *Sbf*I restriction enzymes and an individual barcode consisting of 6 bp. The libraries were paired end sequenced (100 bp reads) on one lane of an Illumina Hiseq 2000 platform in the Bauer Core Facility at Harvard University.

Bioinformatics pipeline

We discarded the paired read to simplify downstream data processing and checked the quality of the single end raw sequencing Illumina reads using FastQC (v.0.11.5; Babraham Bioinformatics; https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Using the ddRAD pipeline created by DaCosta and Sorenson (2014; https://github.com/BU-RAD-seq/) we first de-multiplexed sequences based on their unique barcodes. For each individual we condensed (while maintaining a count) identical sequences and then filtered low quality, unique sequences using the UCLUST function in USEARCH v5 (Edgar 2010). Condensed and filtered reads across all samples were then clustered at an 85% identity level using UCLUST. The highest quality sequence from each cluster was compared to the closest available reference genome (killdeer *Charadrius vociferous,* Zhang *et al.* 2014) using BLASTN (Altschul *et al.* 1990) and we combined clusters with highly similar hits. Sequences within each cluster were aligned using MUSCLE v3 (Edgar 2004a, b). Finally, genotypes were determined for all individuals using the python script *RADgenotypes.py*.

We flagged clusters which had i) fewer than three reads, ii) uneven allele ratios with the minor allele having a lower proportion than 0.3, and iii) extra reads appearing with a proportion ≥ 0.1 implying the presence of more than two alleles. Clusters were removed if they i) were flagged in more than five samples, ii) that had a length < 19 bp, iii) had a median depth of less than 9.5, or iv) had more than five samples with missing data. Clusters with potential alignment errors (see manual at http://github.com/BU-RAD-seq/ddRAD-seq-Pipeline) were also removed, as were clusters with high numbers

of SNPs, > 4 bp indels, skewed heterozygosity ratios (> 5) and very high read depths (> 800) across individuals, since these were indicative of multiple alignment matches.

Z chromosome and W chromosome SNPs

We identified SNPs on the Z chromosome using a two-step method. Firstly, we compared mean read depths for males and females in each of the 1444 polymorphic clusters with the underlying expectation that female depth should be about half that of males for loci on the Z chromosome. Clusters with a female to male average depth ratio between 0.4 and 0.65 were then checked for alignment to the Z chromosome of the killdeer genome (Zhou *et al.* 2014) using our BLASTN results. If both read depth and alignment methods suggested Z-linkage then we assigned the cluster to the Z chromosome dataset. All other clusters were assigned to an "autosomal dataset" after removing a single polymorphic cluster that was only present in females (i.e., zero reads recovered for all male samples), suggesting it was located on the W chromosome.

For the Z chromosome and autosome datasets we retained one biallelic SNP per cluster to minimise linkage. Finally, to counter false signals of population expansion due to an excess of minor alleles (Marth *et al.* 2004) that could have resulted from sequencing errors, and to reduce potential biases in Bayesian clustering solutions (Linck and Battey 2017), we removed loci in which the minor allele count was one in any of the populations unless it was present in one further population. For all analyses, the Z-linked SNP dataset only contained (diploid) males, whereas, the autosomal SNP, microsatellite, and mtDNA datasets included all individuals.

Genetic diversity

We used Arlequin v3.5 (Excoffier and Lischer 2010) to calculate pairwise population differentiation (F_{ST}), genetic diversity (H_{exp}, number of polymorphic markers) in mtDNA (F_{ST}), microsatellite (F_{ST}, H_{exp}) and SNP (F_{ST}, H_{exp}) datasets. We calculated microsatellite allelic richness in MSA v4.05 (Dieringer and Schlötterer 2003) and the inbreeding coefficient (F_{IS}) across all loci for microsatellites and SNPs using the *adegenet* package (Jombart 2008) for R. We calculated per site Watterson's theta (θ_W) across the quality filtered ddRAD dataset (including monomorphic and polymorphic clusters) in PopGenome (Pfeifer *et al.* 2014). We used DNAsp v6 (Librado and Rozas 2009) to calculate haplotype and nucleotide diversity of mtDNA. We calculated diversity statistics separately for all sampling sites, subspecies and nuclear genetic clusters (hereafter demes), the latter identified with STRUCTURE (see below and Figure 3.2).

Population structure

To determine genetic structure with SNPs and microsatellites we performed Bayesian cluster analysis using the program STRUCTURE v2.3.4 (Pritchard *et al.* 2000), automated for command line usage with StrAuto (Chhatre and Emerson 2017). We tested *K* values between one and five for 1 million repetitions and a burn-in of 250,000 repetitions with 20 replicates for each level of *K*. Due to suspected low population differentiation, we ran the program with and without the LOCPRIOR model (Hubisz *et al.* 2009). We merged runs for each *K* (with and without LOCPRIOR separately) using Clumpak (Kopelman *et al.* 2015) implemented through Pophelper (Francis 2017). We determined the most biologically relevant *K* value by inspecting the bar plots grouped according to distance between sampling sites and subspecies, Delta K (Evanno *et al.* 2005) and the Ln Pr(X|K) values, as recommended by Janes *et al.* (2017).

As STRUCTURE can be sensitive to minor allele filtering (Linck and Battey 2017) we additionally performed discriminant analysis of principle components (DAPC) using the package a*degenet* (Jombart 2008, Jombart *et al.* 2010), implemented in R. To evaluate the Bayesian clustering results we set up DAPC models using the *find.clusters* function with Bayesian information criterion (BIC) and also for K=4, to compare with STRUCTURE cluster outcomes. For the mtDNA D-loop sequences, we created a haplotype network using the Templeton, Crandall and Sing (TCS, Templeton *et al.* 1992) method implemented in PopArt (Leigh and Bryant 2015), to visually inspect the distribution of haplotypes across subspecies and demes.

Isolation by distance

To test for isolation by distance we assessed the correlation between geographic and genetic distances using Mantel tests with 9999 permutations in the R package *adegenet* (Jombart 2008). We calculated individual pairwise genetic distances from microsatellites and SNPs as 1 - kinship coefficient (Loiselle et al 1995) estimated using SPAGeDi v1.5 (Hardy and Vekemans, 2002). For mtDNA sequences, we calculated genetic distances using the Maximum Composite Likelihood method implemented in MEGAx (Tamura *et al.* 2004, Kumar *et al.* 2018). We then created Euclidean distance matrices for geographic distances using GenALEx 6.501 (Peakall and Smouse 2012). We performed these analyses across all populations and separately within subspecies *nivosus*, which was sampled at multiple breeding sites (Figure 3.1).

Demography

We used the microsatellite dataset to investigate past effective population size (N_e) changes using *Bottleneck* v1.2.02 (Piry *et al.* 1999) and Msvar v1.3 (Beaumont 1999, Storz and Beaumont 2002). *Bottleneck* tests for heterozygosity-excess from the observed number of alleles under the assumption that after a recent bottleneck there is higher heterozygosity under Hardy-Weinberg equilibrium than under a mutation drift model (Cornuet and Luikart 1996). We used the Wilcoxon signed-rank test implemented in *Bottleneck* to test for heterozygosity excess. Using Msvar we inferred the demographic history of each deme (western *nivosus*, eastern *nivosus*, *tenuirostris* and *occidentalis*) by comparing three models: bottleneck, expansion and stable population based on the full likelihood, coalescent simulation method. Populations within western *nivosus* were also tested independently because of the controversial status of the Pacific coast segment, however, they produced indifferent results (Figure S3.1). Therefore, we combined these populations to provide deme-wide estimates. The stable population model assumed the same N_e values for N_0 (current) and N_1 (ancestral). For the bottleneck, we specified the priors for N_1 to be wider than at N_o (bottleneck), whereas, we assumed the opposite (priors for N_o wider than N_1) for the expansion model. We set the microsatellite mutation rate (μ) to 5 x 10^{-4} substitutions per site per generation (Peery *et al.* 2012), with a range of 10^{-3} to 10^{-5}. We ran each model with 1 x 10^5 MCMC steps (20,000 burn-in) thinning every 100,000 steps to end with 100,000 thinned steps. We performed multiple runs with large variances on priors (Table S3.1) to influence posterior distributions as little as possible. We assessed convergence of the MCMC simulations for each model with the Gelman and Rubin's diagnostic (Gelman and Rubin 1992, Brooks and Gelman 1998) calculated using the *Boa* package v1.1.7 (Smith 2007) in R statistical software.

Table 3.1. Genetic diversity of four snowy plover (*Charadrius nivosus*) populations using four genetic markers (number of markers): autosomal SNPs and Z-linked SNPs from ddRAD sequencing, microsatellites, and mtDNA. Shaded cells indicate populations/subspecies with genetic data for all four markers. * males only; *r* = allelic richness; N° poly = number of polymorphic loci; h = haplotype diversity; H_e = expected heterozygosity; F_{IS} = inbreeding coefficient; π =per site nucleotide diversity. Standard deviations are presented in brackets.

				mtDNA (424 bp mtDNA D-loop)				Autosomal microsatellites (15)					ddRAD autosomal SNPs (798)				ddRAD Z* SNPs (65)			
Subspecies	**Sampling site (pop label)**	**Lat**	**Long**	**N**	**N° poly**	**h**	**π**	**N**	**N° poly**	***r***	**H_e**	**F_{IS}**	**N**	**N° poly**	**H_e**	**F_{IS}**	**N**	**N° poly**	**H_e**	**F_{IS}**
nivosus	Utah, USA (UTA)	41.09	-112.13	32	5	0.65 (0.01)	0.002 (0.0004)	25	13	2.5 (1.2)	0.37 (0.27)	0.01 (0.16)	8	419	0.24 (0.20)	-0.038 (0.31)	3	19	0.15 (0.22)	-0.16 (0.40)
	Monterey bay, USA (MT)	36.74	-121.80	4	1	0.50 (0.07)	0.001 (0.0006)	0	-	-	-	-	1	191	NA	NA	1	11	NA	NA
	Ceuta, Mexico (CEU)	23.90	-106.95	10	1	0.477 (0.02)	0.001 (0.0003)	25	12	2.5 (1.1)	0.39 (0.26)	-0.02 (0.24)	9	505	0.22 (0.19)	-0.004 (0.23)	4	28	0.19 (0.22)	-0.04 (0.24)
	Nayarit, Mexico (NAY)	22.43	-105.59	0	-	-	-	8	11	2.4 (1.1)	0.33 (0.26)	-0.01 (0.27)	0	-	-	-	-	-	-	-
	Texcoco, Mexico (TEX)	19.54	-99.00	0	-	-	-	23	12	2.3 (1.1)	0.35 (0.26)	0.04 (0.17)	0	-	-	-	-	-	-	-
	San Quintin, Mexico (SAQ)	30.39	-115.88	0	-	-	-	22	12	2.4 (1.2)	0.36 (0.27)	0.02 (0.14)	0	-	-	-	-	-	-	-
	UTA, CEU, MT (combined)	-	-	46	6	0.61 (0.05)	0.002 (0.0003)	50	14	2.8 (1.3)	0.41 (0.25)	0.01 (0.12)	18	549	0.23 (0.18)	-0.01 (0.22)	8	32	0.17 (0.19)	0 (0.34)
Florida (*nivosus*)	Florida, USA (FLO)	30.29	-86.73	29	2	0.43 (0.10)	0.001 (0.0002)	43	12	2.2 (0.8)	0.41 (0.17)	0.08 (0.12)	10	480	0.22 (0.20)	0.02 (0.32)	6	28	0.15 (0.20)	0.01 (0.41)
tenuirostris	Cabo Rojo, Mexico (CAR)	17.97	-67.19	23	7	0.67 (0.10)	0.004 (0.0008)	13	10	1.8 (0.8)	0.42 (0.17)	-0.13 (0.18)	9	297	0.14 (0.20)	-0.02 (0.31)	4	6	0.04 (0.14)	-0.45 (0.34)
occidentalis	Paracas, Peru (PAR)	-13.86	-76.27	22	4	0.40 (0.12)	0.0016 (0.0005)	18	11	2.2 (0.7)	0.40 (0.19)	-0.012 (0.20)	10	244	0.12 (0.19)	-0.02 (0.32)	6	18	0.11 (0.18)	-0.09 (0.41)

Table 3.1 Continued next page for mtDNA only

Table 3.1. Continued for mtDNA only

Subspecies	Sampling site	Lat	Long	mtDNA (424 bp mtDNA D-loop) N	N° poly	h	π
nivosus	all locations	NA	NA	152	15	0.696 (-0.025)	0.00234 (0.00018)
occidentalis	all locations	NA	NA	30	8	0.777 (-0.063)	0.00564 (-0.00058)
tenuirostris	all locations	NA	NA	22	3	0.398 (-0.122)	0.00166 (-0.00052)

To examine population demography with the autosomal SNPs we first generated recent migration estimates between demes using a modified version of BayesAss v 3 (Wilson and Rannala 2003). The modified version (available here: https://github.com/smussmann82/BayesAss3-SNPs) allows the analysis of large SNP datasets. Initially, we explored several migration parameter combinations (*m* 0.1-0.6, *a* 0.1-0.6, *f* 0.1-0.5) with short (default) numbers of MCMC steps, burn-in, and sampling values. Once the acceptance rates fell between 20% and 60% (Rannala 2015) we conducted two longer runs with different seeds, with the following parameter combination: *m* 0.4, *a* 0.6, *f 0.4,* for 1 x 10^7 MCMC steps, 1 x 10^6 burn-in and sampling every 500 iterations. We visualised the results and determined the convergence of the two runs using TRACER v1.7 (Rambaut *et al.* 2018). Results of the two runs were averaged to provide migration rate estimates.

We then applied Approximate Bayesian Computation (ABC, Beaumont *et al.* 2002) in ABCtoolbox v2 (Wegmann *et al.* 2010) to compare our empirical data to simulated results from three different demographic models (stable, bottleneck and expansion). ABC was conducted for single demes independently: western *nivosus* (California, Utah and Ceuta combined), eastern *nivosus* (Florida), *occidentalis* and *tenuirostris*.

In brief, ABC uses the following logic (reviewed by Beaumont, 2010). First, summary statistics are calculated from the empirical dataset in addition to a set of simulated datasets created based on pre-defined demographic models (involving e.g. population divergence, bottlenecks, expansions, migration). Second, summary statistics from the empirical dataset are compared with those from the simulated datasets to reveal the best fitting demographic model. Finally, differences between the empirical and best fitting model are used to predict the posterior distributions of model parameters (e.g. past and current effective population size, time of divergence, Beaumont *et al.* 2002).

We specified wide priors to capture variation in parameter estimations (Table S3.2), applied uniform prior distributions, and modelled effective population sizes with $\log_{10}$ transformed values. We used a SNP array approach, whereby our dataset contained 798 independent autosomal loci (see SNP filtering results section). To simulate an initial 10^5 datasets for model optimisation we used fastsimcoal v2.6 (Excoffier and Foll 2011; Excoffier *et al.* 2013). We computed summary statistics for each dataset (simulated and observed SNPs) with the console version of Arlequin v3.5.2.2., *arlsumstat* (Excoffier and Lischer 2010). To infer the best fitting demographic model to our observed dataset we calculated the Euclidean distance between observed and simulated datasets, and carried forward the closest 1,000 simulations. For each model we used the built-in general linear

post-sampling adjustment step within ABCtoolbox to calculate posterior probabilities and marginal densities. We evaluated the best fitting demographic scenario by pairwise comparisons of the marginal densities and *p* values of each model (Wegmann *et al.* 2010). We computed Spearman's Rho statistics to assess pairwise correlation between summary statistics, and removed those that were highly correlated before model comparisons. The uncorrelated summary statistics used in model comparisons were the total number of alleles over loci and the mean heterozygosity. We considered models with *p*-values greater than 0.05 as possible and the best fitting demographic scenario was required to have a Bayes Factor (BF) $\geqq 3$. To produce final posterior parameter values we repeated the best fitting models with 10^6 simulations with the closest 10^3 retained for model fitting and posterior estimations. We tested for stable, expansion or bottleneck models within the last 1,000 years as this focused our analysis on the period of intensive human induced change to coastal habitats (Lotze *et al.* 2006; Spatz *et al.* 2017). The generation time for ABCtoolbox and Msvar was set a 1 year based on snowy plover field observations (e.g. Eberhart-Phillips *et al.* 2017).

Where referred to, we used R statistical software v3.5.0 (R development core team).

Results

SNP generation with ddRAD

We obtained a total of 62.5 million sequence reads from 47 individuals. After initial filtering and de-multiplexing, read coverage ranged from 0.46-1.4 million reads per individual with a mean of 1.1 (s.d. 0.15) million reads. From these we identified 23,024 ddRAD clusters, 4,582 clusters passed quality filters and of these, 1,444 clusters contained between 1-4 polymorphic loci. We removed 106 of these clusters with putative linkage to sex chromosomes, 65 of these were confidently Z-linked and used as the "Z dataset". The remaining 1,338 autosomal clusters included 1,706 SNPs, whereas, the 65 Z chromosome clusters included 84 SNPs. The final autosomal, unlinked dataset consisted of 798 SNPs and the Z chromosome dataset contained 65 SNPs.

Genetic diversity

Per site genetic diversity (Watterson's theta, θ) across all quality filtered ddRAD clusters was low overall but highest in subspecies *nivosus* (Utah: 0.000844, Ceuta: 0.000804 and Florida: 0.000729), followed by *tenuirostris* (0.00048) and *occidentalis* (0.00045). Similarly, microsatellite mean allelic richness was low and ranged from 1.8 in *tenuirostris* up to 2.5 in *nivosus* populations (Utah and Ceuta) (Table 3.1). Within 233 individuals distributed across the range of the snowy plover (n=233), we identified 25 haplotypes of a 424 bp section D-loop mtDNA region. MtDNA haplotype and nucleotide diversity was highest in *occidentalis*, followed by *nivosus,* and *tenuirostris* (Table 3.1). No substantial level of inbreeding was detected, however, comparing between populations, Florida consistently had the greatest mean inbreeding coefficient ranging between 0.02 (s.d. 0.32) in autosomal SNPs to 0.08 (s.d. 0.12) in microsatellites (Table 3.1).

Population structure

Microsatellites and autosomal SNPs

Microsatellite and SNP datasets split *nivosus* populations from the remaining two subspecies where $K = 2$ in the Bayesian structure analysis (data not shown). At $K = 3$, microsatellites recovered the updated delineation of the three subspecies according to Funk *et al.* (2007) but showed a high proportion of admixture between *nivosus* and

tenuirostris unless prior location information was included (Figure 3.2). In contrast, autosomal and Z-linked RAD loci indicated little to no admixture between the subspecies at K = 3 (Figure 3.2). The best models of the STRUCTURE analysis suggested four separate demes: *tenuirostris*, *occidentalis*, eastern *nivosus* (Florida) and western *nivosus* (all other *nivosus* populations). The use of location priors improved the assignment to demes for microsatellites and did not change the assignment of individuals for autosomal, nor Z-linked SNPs (Figure 3.2). The Z-linked SNP dataset corroborated the autosomal SNP and microsatellite results at K = 2 (data not shown) and K = 3 but not for K = 4 (Figure 3.2). Using the autosomal and Z chromosome dataset, DAPC analysis largely corroborated the STRUCTURE demes by distinguishing between the three subspecies (Figure 3.3A, B, C). In addition, when forcing the data into four demes, eastern and western snowy plovers were split for the autosomal SNPs (Figure 3.3D) but not for Z-linked SNPs, nor microsatellites (Figure 3.3C, E). With microsatellite loci, the separation of *tenuirostris* and *nivosus* was marginal (Figure 3.3B) and the DAPC failed to detect fine scale structure between eastern and western snowy plovers (Figure 3.3B, E).

mtDNA

According to mtDNA analysis, subspecies were not reciprocally monophyletic. We observed multiple shared haplotypes between *nivosus* and *tenuirostris,* whereas, only one haplotype was shared between *occidentalis* and *tenuirostris* (Figure 3.4). Haplotypes from *occidentalis* and *tenuirostris* clustered together (Figure 3.4). Eastern *nivosus* showed one exclusive haplotype, whereas, we detected twelve exclusive haplotypes in western *nivosus,* three in *occidentalis,* and four in *tenuirostris* (Figure 3.4).

All markers

Between subspecies we found high and significant pairwise population differentiation with all markers (Table 3.2). Mitochondrial DNA gave the highest differentiation estimates across all nuclear demes (Φ_{ST} from 0.03 to 0.79). For every deme comparison, Z-linked SNPs had greater F_{ST} values in comparison with those calculated using autosomal SNPs (Table 3.2). Subspecies *occidentalis* was most differentiated from all other demes (Table 3.2). Eastern versus western *nivosus* demes showed significant differentiation (F_{ST} = 0.04-0.11) at all markers with the exception of mtDNA (Φ_{ST} = 0.03).

Isolation by distance

We found significant isolation by distance patterns for three of the four markers when including all subspecies and within *nivosus* populations only ($p<0.05$; Figure 3.5). Using mtDNA, isolation by distance was only significant when including all subspecies (Figures 3.5i, B and 3.5ii, B). Across the species as a whole, with nuclear markers, isolation by distance was weakest for microsatellites and strongest with Z-linked SNPs. Within *nivosus* only, we detected the strongest isolation by distance with autosomal SNPs, followed by Z-linked SNPs, and a weak but significant isolation by distance relationship with microsatellites (Figure 3.5ii).

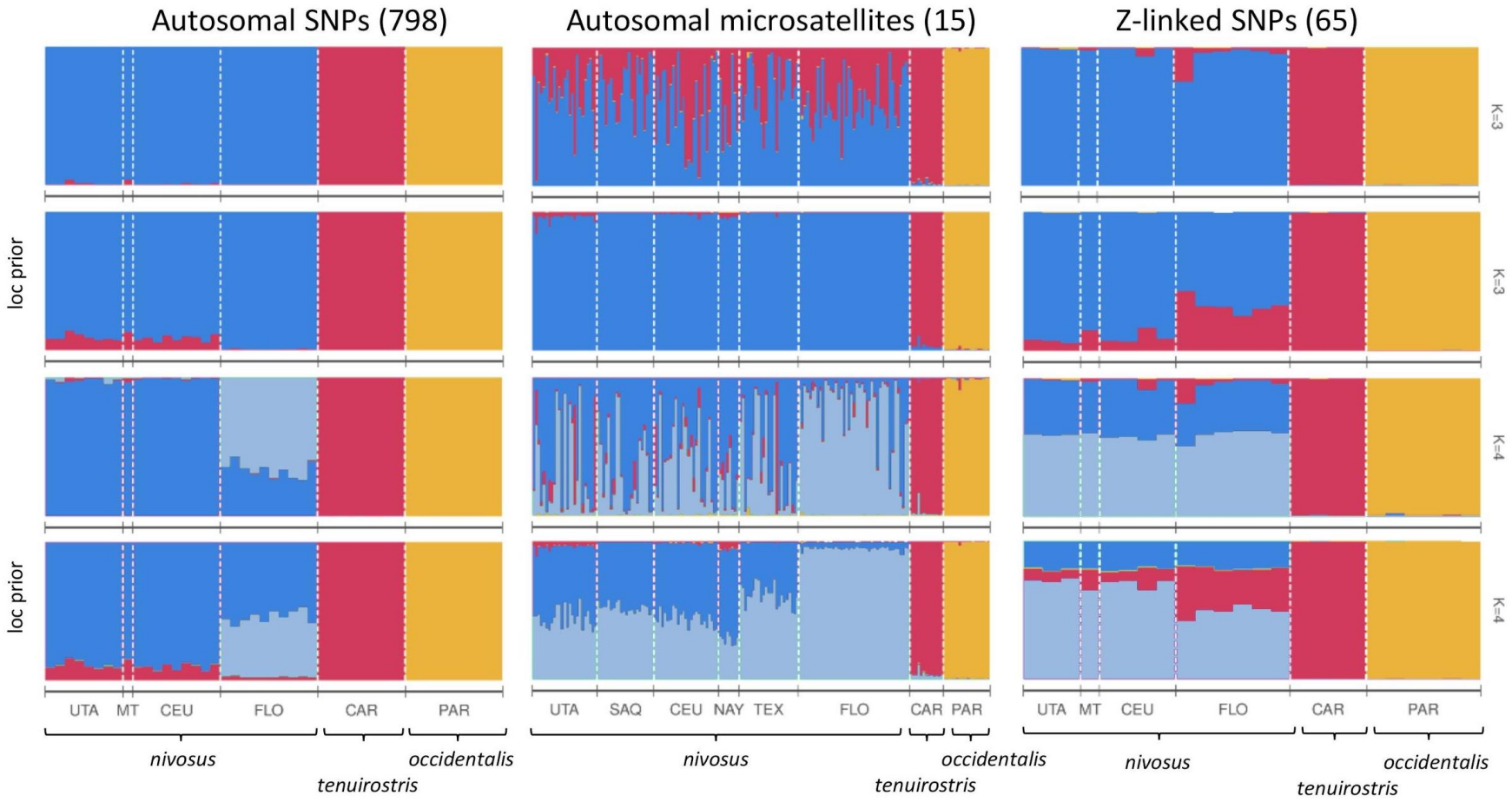

Figure 3.2. Results of Bayesian clustering analyses with and without location priors of snowy plovers (*Charadrius nivosus*) using three genetic markers (798 autosomal SNPs; 15 autosomal microsatellites and 65 Z-linked SNPs) estimated by STRUCTURE (Pritchard *et al.* 2000). The most likely clustering solutions, $K = 3$ and $K = 4$ are shown. See Table 3.1 for population codes and respective subspecies are indicated below plots.

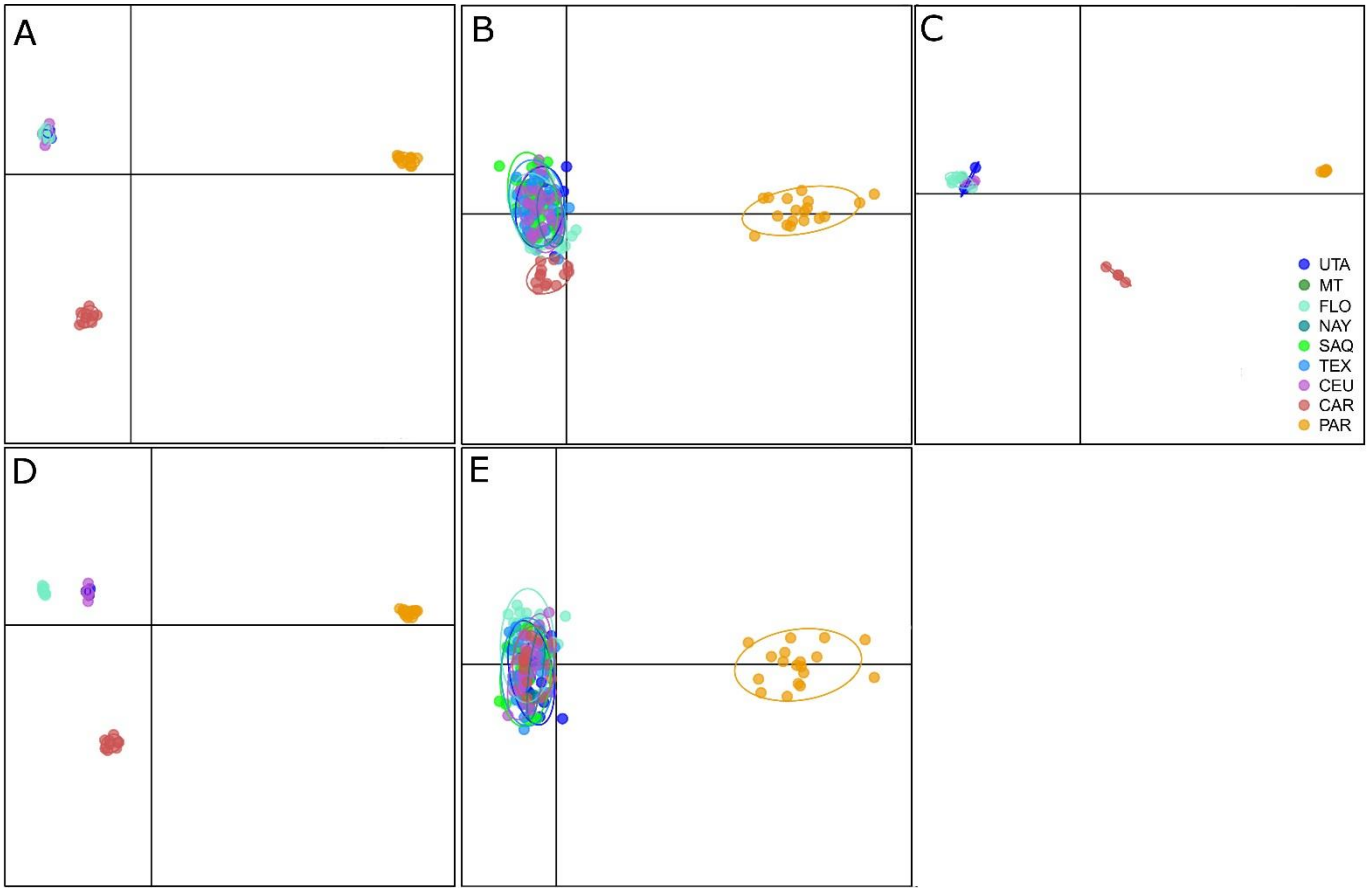

Figure 3.3. Discriminant analysis of principle components (DAPC) of snowy plover (*Charadrius nivosus*) using three genetic markers: 798 autosomal SNPs (A and D), 15 microsatellites (B and E) and 65 Z-linked SNPs (C). A-C = scatterplots created using the number of clusters according to Bayesian Information Criterion (BIC; autosomal SNPs: 3 clusters, autosomal microsatellites: 7 clusters, Z-linked SNPs: 4 clusters), and D-E shows DAPC when forcing four clusters. Population codes are presented in Table 3.1.

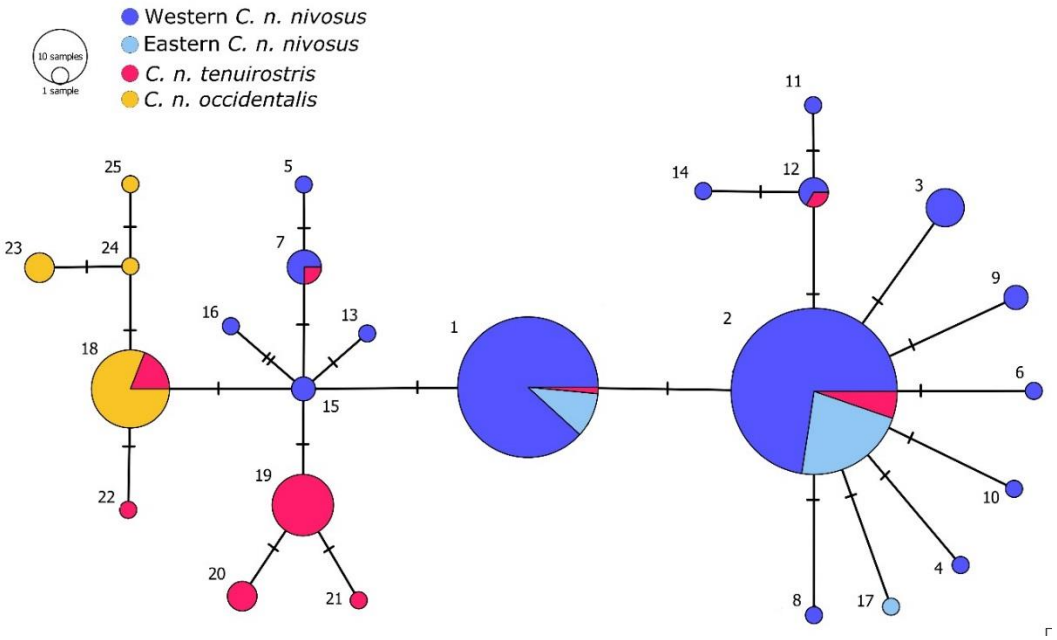

Figure 3.4. Haplotype joining network created using the statistical parsimony method (TCS; Templeton *et al.*, 1992) of snowy plover (*Charadrius nivosus*) D-loop mtDNA sequences. Samples were collected from localities representing all three subspecies. Colour coding represents nuclear clusters, see Figures 3.1 and 3.2.

Table 3.2. Pairwise F_{ST} (and Φ_{ST} mtDNA) comparisons between nuclear demes of snowy plover (*Charadrius nivosus*), see Figure 3.2 for population clustering. Significant values are indicated in bold. Blue = microsatellites; red = mtDNA; green = Z-linked SNPs; yellow = autosomal SNPs

	western *nivosus*		**eastern *nivosus***		***tenuirostris***	***occidentalis***
western *nivosus*						
eastern *nivosus*	**0.04**	0.03				
	0.11	**0.06**				
tenuirostris	**0.12**	**0.54**	**0.15**	**0.47**		
	0.35	**0.22**	**0.30**	**0.27**		
occidentalis	**0.43**	**0.71**	**0.47**	**0.79**	**0.47**	**0.48**
	0.45	**0.38**	**0.49**	**0.44**	**0.66**	**0.58**

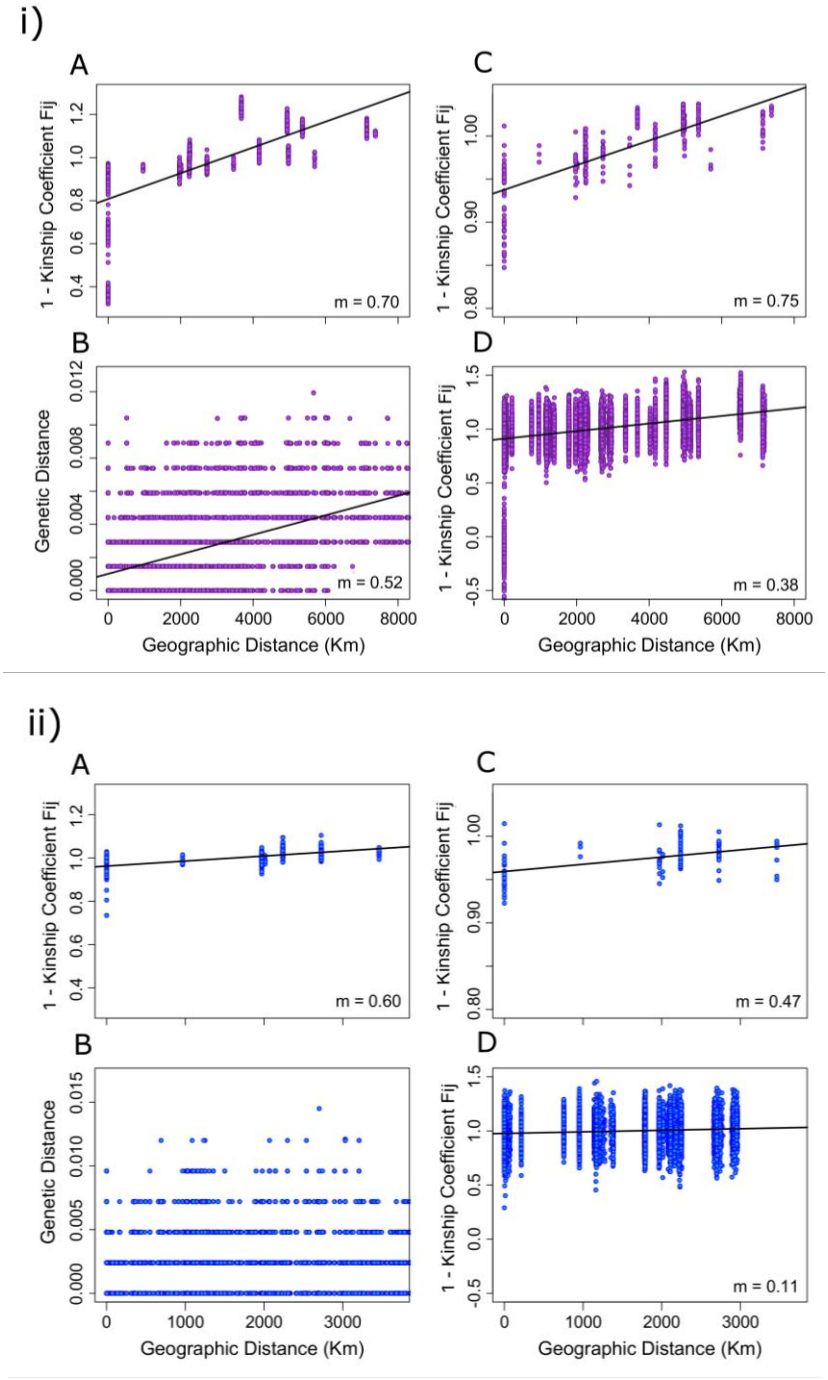

Figure 3.5. Pairwise individual-based isolation by distance (IBD) across snowy plover (*Charadrius nivosus*) populations estimated from four genetic markers: 798 autosomal SNPs from ddRAD sequencing (A), mtDNA (B), 65 Z-linked SNPs (C), and 15 microsatellites (D). i) shows IBD across all three subspecies, whereas ii) is IBD within *C. n. nivosus* only. Mantel test statistic = m, regression lines are provided where significant IBD ($p<0.05$) was found.

Demography

Overall our demographic analysis provided strong evidence for population bottlenecks in all subspecies, with an effective population decline of over 97% in all populations during the past 1,000 years. Specifically, Msvar predicted bottlenecks occurring between 59 and 434 years ago (YA) (Figure 3.6, 3.7; Table 3.3, Table S3.3) when the effective population size decreased from an average of 5,988 (s.d. 378) to 34 (s.d. 18). The largest effective population decline was found in western *nivosus* and *tenuirostris* and the smallest in *occidentalis* (Figure 3.6; Table 3.3). Supporting microsatellite Msvar results, the population bottleneck model best fitted our observed autosomal SNP datasets of each deme according to ABC analysis (Table S3.4). ABC predicted the bottlenecks occurred most recently in *occidentalis* and *tenuirostris* (212 YA and 242 YA respectively) compared to eastern and western *nivosus* (759 YA and 764 YA respectively) (Figure 3.7; Table 3.3). ABC showed the largest effective population size decline was in *tenuirostris* (from 36,138 to 333) and the smallest in western *nivosus* (from 2,313 to 70; Figure 3.6; Table 3.3). *Tenuirostris* suffered the greatest population size decline according to all three methods, and was the only deme to show support for a bottleneck based on heterozygosity excess with the program *Bottleneck* (Wilcoxcon test: p= 0.04).

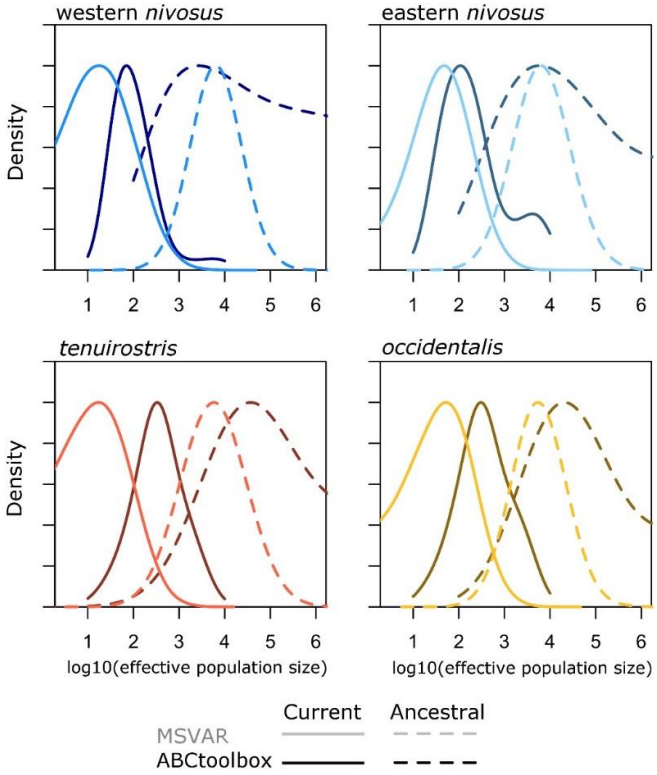

Figure 3.6. Effective population size (current and ancestral) for four population demes of snowy plover (*Charadrius nivosus*) estimated by Msvar (Beaumont 1999, Storz and Beaumont 2002) and ABCtoolbox (Wegmann *et al.* 2010). Density is normalised for direct comparison of methods. ABCtoolbox posteriors are restricted by prior parameter values. Effective population is reported in $\log_{10}$ scale.

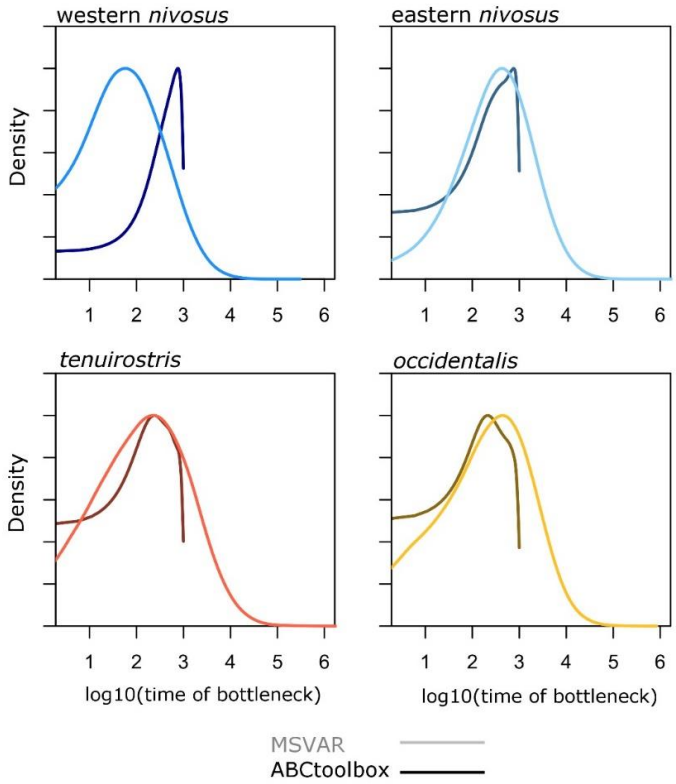

Figure 3.7. Estimated time of population bottlenecks for four population demes of snowy plover (*Charadrius nivosus*). Estimated by Msvar (Beaumont 1999, Storz and Beaumont 2002) and ABCtoolbox (Wegmann *et al.* 2010). Density is normalised for direct comparison of methods. ABCtoolbox posteriors are restricted by prior parameter values. Time of bottleneck is in $\log_{10}$ scale.

Table 3.3. Effective population size (N_e) and time of bottleneck of snowy plover (*Charadrius nivosus*) demes estimated with Msvar (Beaumont 1999, Storz and Beaumont 2002) and ABCtoolbox (shaded cells, Wegmann *et al.* 2010) with 95% highest posterior densities. Inf = over 10^6 individuals. Msvar values are derived from the bottleneck scenario.

	Current N_e		**Ancestral N_e**		**Time of bottleneck**	
	Mode	HPD95%	Mode	HPD95%	Mode	HPD95%
C. n. tenuirostris	333	27-4,659	36138	745-inf.	242	26-940
	18	<1-417	5888	229-12,3027	228	<1-10,715
C. n. occidentalis	300	26-5,542	20971	567-inf.	212	21-930
	51	<1-977	5495	457-79,433	434	<1-12,023
C. n. nivosus (western)	70	11-1,012	2613	142-inf.	764	152-1,000
	18	<1-447	6310	589-74,131	59	<1-2,138
C n. nivosus (eastern)	110	16-6,593	6010	174-inf.	759	86-990
	47	<1-1,445	6310	447-112,202	422	1-16,982

We found low but significant levels of recent migration (0.02-0.04) between all four demes from BAYESASS analyses (Table S3.5). There was one notable exception of high migration (0.27, s.d. 0.03) indicating substantial gene-flow from western to eastern *nivosus* populations. However, given the low sample sizes and the weak population differentiation between Florida and *nivosus* F_{ST} ~0.05, these results should be interpreted with caution (Meirmans 2014).

Discussion

Here we present a thorough re-evaluation of the snowy plover subspecies and population delineation based on a comprehensive assessment of genetic differentiation using four different genetic markers. Generally, we find support for the current snowy plover subspecies delineations as proposed by Funk *et al.* (2007). The subspecies divergence we describe here with microsatellite (F_{ST} 0.12-0.47), SNP (F_{ST} 0.22 – 0.57) and mtDNA markers (F_{ST} 0.46-0.74) in snowy plovers is similar to other species of plover, for example, the piping plover (*Charadrius melodus*, Miller *et al.* 2010) and the chestnut banded plover (*Charadrius pallidus*, dos Remedios *et al.* 2017). However, snowy plover subspecies are more distinct compared to the subspecies of other shorebirds for example dunlin, *Calidris alpina* (Miller *et al.* 2015) and redshank, *Tringa totanus* (Ottvall *et al.* 2005). This level of differentiation is also greater than that between the white-faced plover (*Charadrius alexandrinus dealbatus*) and Kentish plover (*Charadrius alexandrinus*, F_{ST} = 0.01, Rheindt *et al.* 2011) which are considered separate species by some authorities based on phenotypic differences (del Hoyo *et al.* 2018, but see Clements *et al.* 2017, Gill and Donsker *et al.* 2018).

By combining new data with those from two previous published papers (Funk *et al.* 2007, D'Urban Jackson 2017) we substantially increase the number of genetic markers, individuals, and *nivosus* sampling locations. The SNP results corroborate previous findings (Funk *et al.* 2007, D'Urban-Jackson *et al.* 2017) that the Florida population is closer related to other *nivosus* populations, rather than an alternative delineation as part of *tenuirostris* (American Ornithologist's Union 1957). However, our results, especially from SNP markers, suggest that this population is part of another deme, eastern snowy plovers, that is genetically differentiated from western snowy plovers *nivosus* at nuclear loci. This supports the current state-wide protection of Florida snowy plovers as a separate management unit. However, we lack samples from breeding populations in the interior United States and west of Florida to fully characterise the boundaries of eastern and western *nivosus* demes. Therefore, we suggest the previous American Ornithologist's Union delineation for mainland *nivosus* and *tenuirostris* plovers as boundaries of these demes, which is based on plumage differences (American Ornithologist's Union 1957), until further studies are completed. This delineation refers to mainland snowy plovers east of Louisiana as eastern snowy plovers. Estimates of recent migration suggest that eastern snowy plover populations receive gene-flow from western snowy plovers, but there is less gene-flow in the opposite direction. This bias maybe the result of wintering western migrants occasionally remaining to breed in the distribution of the eastern snowy plover.

Snowy plovers have been the subject of several movement and demographic studies, however, so far these have not encompassed the entire range (Warriner *et al.* 1986,

Stenzel *et al.* 1994, 2007, 2011, Eberhart-Phillips and Colwell 2014, Aiello-Lammens and Akcakaya 2016, Cruz-López *et al.* 2017, Eberhart-Phillips *et al.* 2017). Therefore, the long distance dispersal of this species remains largely unknown, with no available evidence so far supporting a unidirectional movement from western *nivosus* populations to Florida. Nevertheless, gene-flow from the larger western to the smaller eastern *nivosus* populations observed in the present study may provide a crucial bolstering effect against population declines in this small and vulnerable population (Lamonte *et al.* 2002).

Despite the differentiation between the eastern and western *nivosus* populations we observed only a weak isolation by distance effect within the *nivosus* subspecies. This is remarkable given the large distance between sites sampled (>3000km). Weak clinal variation structure or genetic homogeneity between populations is common in shorebirds, for example, Temminck's stint *Calidris temminckii* (Rönkä *et al.* 2012), ruff, *Philomachus pugnax* (Verkuil *et al.* 2012), mountain plover *Charadrius montanus* (Oyler-McCance *et al.* 2008) and the closely related Kentish plover, *C. alexandrinus* (Küpper *et al.* 2012). Interestingly, across the snowy plovers as a whole, we find Z-linked markers provide the strongest isolation by distance pattern compared to moderate levels in autosomal microsatellites and mtDNA, suggesting male philopatry. In support this finding, Z-linked SNPs produced higher pairwise F_{ST} levels compared to autosomal markers, therefore, taken together these results could suggest female biased dispersal. Female biased dispersal has been observed in snowy plovers (Stenzel *et al.* 1994, 2007, Paton and Edwards 1996, Colwell *et al.* 2007, Pearson and Colwell 2014) may be explained by the "dispersal-to-mate" hypothesis which predicts that polyandrous females disperse during a breeding season to increase breeding success (Küpper *et al.* 2012, D'Urban Jackson *et al.* 2017). However, female biased dispersal is common across avian families regardless of mating system (Greenwood 1980, Clarke *et al.* 1997). Therefore, further analysis is needed to confirm or reject support for the "dispersal-to-mate" hypothesis. Furthermore, mtDNA produced the highest F_{ST} of all markers and it is not considered a suitable marker alone to detect true isolation by distance patterns (Teske *et al.* 2018). The pattern of greater Z chromosome compared to autosome population differentiation that we observe here is a common finding in birds (reviewed by Irwin 2018). High relative Z chromosome divergence has led to the hypothesis that the Z chromosome plays proportionally greater role in intra- and inter specific divergence, termed "Fast-Z" evolution (Mank *et al.* 2007, Meisel and Connallon 2013). However, DAPC and Bayesian cluster analysis with Z-linked SNPs failed to distinguish clearly between eastern and western snowy plovers compared to autosomal loci. We suggest that the conflicting patterns of differentiation and isolation by distance from Z-linked SNPs may reflect the low power of a small number of polymorphic Z chromosome markers (n=65) compared to autosomal markers (n=798).

Accordingly, autosomal polymorphic SNPs corroborate, and add clarity to genetic differentiation between all four demes compared with 15 microsatellites in population structure analyses. Our findings highlight the advantages of using a combined genetic approach when investigating potentially controversial fine scale population structure, especially in high gene-flow species such as the snowy plover (e.g. Ruegg *et al.* 2014, Vendrami *et al.* 2017).

Consistent with previous findings (Funk *et al.* 2007), we find no evidence of genetic differentiation between coastal and interior western *nivosus* populations and no differentiation among coastal Pacific populations. This is not surprising given the high dispersal capabilities of the species (Stenzel *et al.* 1994) and the fact that just one

migrant per generation is required to homogenise a gene pool (Mills and Allendorf 1996). To detect existing fine scale differentiation within this deme, substantially more markers will be required, for example, by using whole genome sequencing (WGS). Combining a WGS approach with improved sampling strategy to encompass the entire range of the species would enable not only the delineation of deme boundaries but also allow for investigation into adaptive variation (Kjeldsen *et al.* 2016).

Supporting previous studies, we found that the current effective population size of this species is low (Funk *et al.* 2007, D'Urban Jackson *et al.* 2017). We predict that this is most likely the result of strong population bottlenecks in all subspecies that have occurred within the last 1000 years. As a result of these bottlenecks, the effective population size of each deme was reduced by at least 97%. Our results from the full likelihood (Msvar, microsatellites) and ABC (ABCtoolbox, autosomal SNPs) approaches corroborated each other well, but differed in the predicted timing of the bottlenecks, particularly within *nivosus* demes. The inconsistent estimation of bottleneck time between ABC and Msvar may have been caused by conflicting demographic trends across the breeding range. The western *nivosus* range spans over large latitudinal and longitudinal gradients (more than 30 and 40 degrees, respectively), and encompasses populations that have experienced growths and contractions. For example, western *nivosus* populations at the Pacific coast declined in the 1980s (Palacios *et al.* 1991), however, due to intensive conservation effort they have stabilised or even increased over the last 20 years based on census results (Mullin *et al.* 2010, Colwell *et al.* 2017, Feucht et al 2017). By contrast, Pacific populations in Mexico appear to be rapidly declining (Galindo-Espinosa and Palacios 2015, Cruz-López *et al.* 2017).

The effective population size based on ABC were consistently higher than those from the Msvar but both method estimates were far lower than the census sizes of breeding snowy plover populations (Thomas *et al.* 2012). For example, the census size of eastern snowy plovers has been estimated between 683-1022 (Thomas et al 2012), whereas, our effective population size estimate was between 47 (Msvar) and 110 (ABC) albeit with wide confidence intervals (CI) of 1-1445 (Msvar) and 16-6593 (ABC). Even more staggering, the western *nivosus* population size was estimated at 18 (Msvar, CI: 1-447) and 70 (ABC, CI: 11-1012), whereas, the census size is over 8000 (Thomas et al 2012). Shafer *et al.* (2015a) found that more markers yielded more power in estimating different demographic models using ABC, with over 50,000 loci being needed for effective population size estimations. The accuracy of ABC compared to other methods in estimating effective population size is largely unknown (Wang *et al.* 2016), and ascertainment bias in SNP arrays can lead to erroneous time estimates (Quinto-Cortés *et al.* 2018). Furthermore, the large confidence intervals of all the estimate parameters should be considered in the when interpreting these results. Taken together we suggest our results indicate a recent substantial reduction in population size across snowy plover populations, however, the effective population size estimates themselves should not be considered reliable.

All of the demographic estimation methods we implemented (ABC, Msvar and *Bottleneck*) indicated the populations size reduction has been most pronounced in subspecies *tenuirostris*. This finding is supported by current population extirpations of *tenuirostris* on Caribbean islands (Brown 2012). *Bottleneck* did not find evidence of a population reduction in *occidentalis* or *nivosus* subspecies, which could be because the program is most suited to detect low magnitude population bottlenecks (Peery *et al.* 2012).

Furthermore, the detection of bottlenecks can rely heavily on the number of markers used (Hoban *et al.* 2013b).

During the past 1000 years, humans have dominated and profoundly altered global ecosystems (Vitousek *et al.* 1997, Barnosky *et al.* 2011, Ceballos *et al.* 2015) especially in coastal and island environments (Lotze *et al.* 2006, Spatz *et al.* 2017). The introduction of predators such as the non-native red fox (*Vulpes vulpes*) and feral cats (*Felis catus*) and in addition to human disturbance of coastal environments have both led to reduced snowy plover breeding productivity and survival (Lafferty 2001, Ruhlen *et al.* 2003, Colwell *et al.* 2007, Webber *et al.* 2013, Dinsmore *et al.* 2017). Therefore, we hypothesise the population size reduction of snowy plovers has resulted from the accumulated impacts of increasingly human modified snowy plover habitats. Estimating demographic histories is becoming more common in conservation genetic studies (Carnaval *et al.* 2009, Palsbøll *et al.* 2013, Shafer *et al.* 2015a, Stoffel *et al.* 2018), however, the use of these methods in prioritizing conservation units has only recently been implemented (Stockwell *et al.* 2013). Here we demonstrate how this information can be incorporated to determine conservation segments of prioritisation for the snowy plover.

Implications for snowy plovers and future work

The low and declining effective population size, and continued threats to snowy plovers indicate that this species remains vulnerable. Our understanding of this species is overwhelmingly reliant on studies from the *nivosus* subspecies, with little dedicated monitoring programs for the *tenuirostris* and *occidentalis* subspecies. The demographic findings we present here and the recent population extirpations occurring in *tenuirostris* (Brown 2012), provide strong justification for increased efforts for the Caribbean subspecies to halt the loss of genetic diversity and adaptive potential (Willi *et al.* 2006). In addition, basic monitoring of *occidentalis* breeding populations, such as those conducted by Wetlands International, are required to obtain reliable information on population size and trends.

Due to the high dispersal capabilities and gene-flow of snowy plovers, future work should use a WGS approach with comprehensive sampling to focus on adaptive diversity in combination with actual movement data (e.g. GPS tagging) and demographic studies. This would allow us to fully characterise the population connectivity and thus, infer conservation management units (Crandall *et al.* 2000, Fraser and Bernatchez 2001, Palsbøll *et al.* 2007, Lowe and Allendorf 2010, Funk *et al.* 2012, Flanagan *et al.* 2017). Snowy plovers offer great opportunities for improving our understanding of mating system evolution and the role this plays in conservation. For example, the number of polyandrous females in a population can increase the genetic diversity (Taylor *et al.* 2014), however, polyandrous behaviour and uniparental care lowers snowy plover chick survival (Cruz-López *et al.* 2017). Future work could disentangle this by combining statistical modelling with empirical data from wild populations.

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Chapter 3 Supplementary Material

Table S3.1. Msvar (Beaumont 1999, Storz and Beaumont 2002) priors and hyperpriors. Columns 3 to 6 show, in a $\log_{10}$ scale, the starting values for the means and variances of the prior distributions for snowy plover (*Charadrius nivosus*) populations. Columns 7 to 10 show the hyperprior distributions for the means and variances (and their means and variances). The same priors were used for all four populations. *g:* generation length; N_0:, current N_e; N_1: ancestral N_e; π: mutation rate per locus per generation; *T:*, time since the population change.

		Prior Distributions				**Hyperprior Distributions**			
Run#	***g***	**log(N_0)**	**log(N_1)**	**log(π)**	**log(T)**	**log(N_0)**	**log(N_1)**	**log(π)**	**log(T)**
1 Stable	2	4 2	4 2	-3.3 1	2 3	4 3 0 0.5	5 3 0 0.5	-3.3 0.5 0 2	2 3 0 0.5
2 Bottleneck	2	4 2	5 2	-3.3 1	2 3	4 3 0 0.5	5 3 0 0.5	-3.3 0.5 0 2	2 3 0 0.5
3 Expansion	2	5 2	4 2	-3.3 1	2 3	5 3 0 0.5	4 3 0 0.5	-3.3 0.5 0 2	2 3 0 0.5

Table S3.2. Prior range values and rules of estimated parameters for three demographic scenarios tested with ABCtoolbox (Wegmann et al. 2010) using simulation datasets produced by fastsimcoal2 (Excoffier *et al*. 2013) to assess effective population size change of snowy plover (*Charadrius nivosus*) demes. All priors are transformed into $\log_{10}$ values. N_e = effective population size. Ancestral population refers to a time point between 1 and 1000 years ago, before the expansion or bottleneck occurred.

Scenario	**Current N_e**	**Ancestral N_e**	**Rules**	**Mutation rate**
Stable	1-4	1-4	-	-6 to -2
Bottleneck	1-4	2-8	Current N_e < Ancestral N_e	-6 to -2
Expansion	1-4	1-3	Current N_e > ancestral N_e	-6 to -2

Table S3.3. Msvar (Beaumont 1999, Storz and Beaumont 2002) values and 95% highest posterior density (HPD) limits of the posterior distributions for the current (N_0) and ancestral (N_1) effective population sizes, and the time since the population size change, in four snowy plover (*Charadrius nivosus*) populations, calculated using BOA v1.1.7.

		Current population size (N_0)		**Ancestral population size (N_1)**		**Time since population change (in years)**	
	Run#	**Mean**	**HPD95%**	**Mean**	**HPD95%**	**Mean**	**HPD95%**
eastern *nivosus*	1 Stable	34	1-912	6310	389-97,724	295	7-10,715
	2 Bottleneck	19	<1-1,445	6457	447-112,202	174	1-16,982
	3 Expansion	29	<1-832	6457	398-95,499	257	4-10,471
western *nivosus*	1 Stable	7	<1-407	6166	550-69,183	31	<1-1,862
	2 Bottleneck	3	<1-447	6457	589-74,131	12	<1-2,138
	3 Expansion	3	<1-501	6457	589-69,183	15	<1-2,138
tenuirostris	1 Stable	4	<1- 398	5370	186-13,4896	56	<1-11,482
	2 Bottleneck	5	<1-417	5623	229-12,3027	74	<1-10,715
	3 Expansion	4	<1-427	5623	223-12,8825	69	<1-7,762
occidentalis	1 Stable	17	<1-871	5623	427-77,625	155	1-9,772
	2 Bottleneck	9	<1-977	5623	457-79,433	83	<1-12,023
	3 Expansion	9	<1-9,772	5754	<1-12,303	89	<1-9,550

Table S3.4. ABCtoolbox (Wegmann *et al.* 2010) demographic scenario model fitting of snowy plover (*Charadrius nivosus*) populations. Shaded cells indicate the best fitting model. All models run for 10^5 simulations unless otherwise stated.

	Scenario	*p* value	marginal density
tenuirostris	stable	0.86	0.52
	bottleneck	0.98	4.72
	bottleneck*	0.98	4.44
	expansion	0.04	0.08
occidentalis	stable	0.68	0.38
	bottleneck	0.97	4.6
	bottleneck*	0.99	4.12
	expansion	0.06	0.07
western *nivosus*	stable	0.97	0.72
	bottleneck	0.76	3.1
	bottleneck*	0.97	4.02
	expansion	0.71	0.27
eastern *nivosus*	stable	0.3	0.92
	bottleneck	1	3.35
	bottleneck*	0.87	4.67
	expansion	0.24	0.13

* run for 10^6 simulations

Table S3.5. Mean posterior migration rate estimates (*m*) and standard deviation (s.d.) between source and destination snowy plover (*Charadrius nivosus*) populations calculated in Bayesass v3 (Wilson and Rannala, 2003) with 798 autosomal SNPs.

Source	Destination	*m*	s.d.
western *nivosus*	*tenuirostris*	0.0257	0.0238
occidentalis	*tenuirostris*	0.0257	0.0236
eastern *nivosus*	*tenuirostris*	0.0255	0.0235
tenuirostris	western *nivosus*	0.0223	0.0209
occidentalis	western *nivosus*	0.0223	0.0208
eastern *nivosus*	western *nivosus*	0.2665	0.0335
tenuirostris	*occidentalis*	0.0222	0.0208
western *nivosus*	*occidentalis*	0.0221	0.0207
eastern *nivosus*	*occidentalis*	0.0444	0.0283
tenuirostris	eastern *nivosus*	0.0167	0.0158
western *nivosus*	eastern *nivosus*	0.0168	0.0159
occidentalis	eastern *nivosus*	0.0166	0.0158

Table S3.6. Snowy plover (*Charadrius nivosus*) mitochondrial D-loop sequences from Funk *et al.* (2007) and their Genbank accession numbers, haplotype number (from this study) and localities (Long = degrees longitude, Lat = degrees latitude).

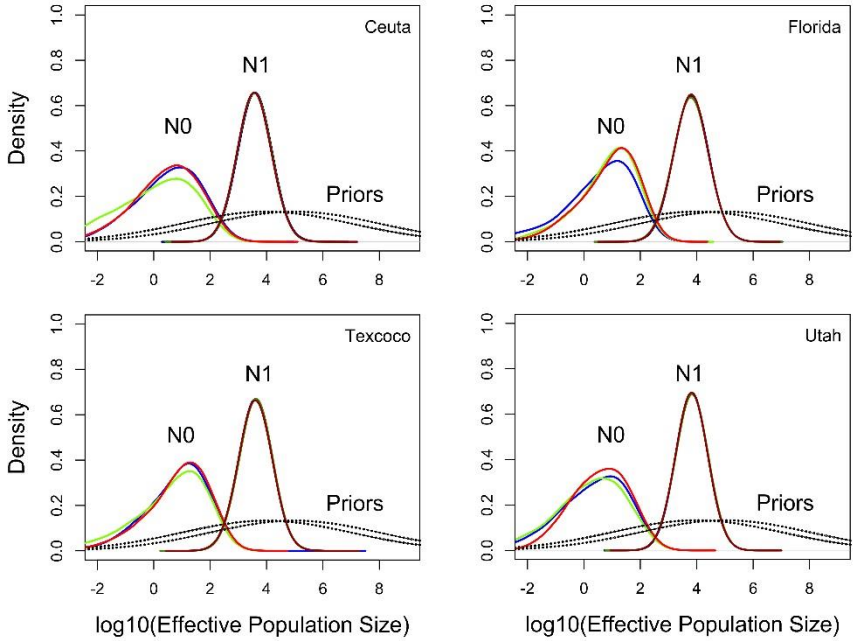
Genbank accession	ID	h	Lat	Long	Genbank accession	ID	h	Lat	Long
EF215532.1	WA1	1	46.75	-124.08	EF215587.1	SCA13	2	36.6	-117.12
EF215533.1	WA2	1	46.75	-124.08	EF215591.1	ORSL2	1	42.63	-120.23
EF215534.1	WA3	1	46.75	-124.08	EF215592.1	ORSL3	2	42.63	-120.23
EF215535.1	WA4	2	46.75	-124.08	EF215593.1	ORSL4	1	42.63	-120.23
EF215536.1	WA5	1	46.75	-124.08	EF215594.1	ORSL5	1	42.63	-120.23
EF215537.1	WA6	3	46.75	-124.08	EF215595.1	ORSL6	1	42.63	-120.23
EF215538.1	WA7	2	46.75	-124.08	EF215596.1	ORSL7	1	42.63	-120.23
EF215539.1	WA8	1	46.75	-124.08	EF215597.1	ORSL8	1	42.63	-120.23
EF215540.1	WA9	2	46.75	-124.08	EF215598.1	ORSL9	11	42.63	-120.23
EF215541.1	WA10	4	46.75	-124.08	EF215599.1	UT1	2	41.09	-112.13
EF215542.1	WA11	1	46.75	-124.08	EF215600.1	UT2	1	41.09	-112.13
EF215543.1	WA12	2	46.75	-124.08	EF215601.1	UT3	2	41.09	-112.13
EF215544.1	WA13	1	46.75	-124.08	EF215602.1	UT4	1	41.09	-112.13
EF215545.1	WA14	5	46.75	-124.08	EF215603.1	UT5	1	41.09	-112.13
EF215546.1	WA15	1	46.75	-124.08	EF215604.1	UT6	2	41.09	-112.13
EF215547.1	ORC1	3	43.55	-124.22	EF215605.1	UT7	1	41.09	-112.13
EF215548.1	ORC2	6	43.55	-124.22	EF215606.1	UT8	2	41.09	-112.13
EF215549.1	ORC3	2	43.55	-124.22	EF215607.1	UT9	1	41.09	-112.13
EF215550.1	ORC4	3	43.55	-124.22	EF215608.1	UT10	2	41.09	-112.13
EF215551.1	ORC5	2	43.55	-124.22	EF215609.1	UT11	1	41.09	-112.13
EF215552.1	ORC6	2	43.55	-124.22	EF215610.1	UT12	12	41.09	-112.13
EF215553.1	ORC7	2	43.55	-124.22	EF215611.1	UT13	2	41.09	-112.13
EF215554.1	ORC8	7	43.55	-124.22	EF215612.1	UT14	2	41.09	-112.13
EF215555.1	ORC9	1	43.55	-124.22	EF215613.1	UT15	2	41.09	-112.13
EF215556.1	ORC10	1	43.55	-124.22	EF215614.1	CO1	1	38.32	-102.7
EF215557.1	ORC11	2	43.55	-124.22	EF215615.1	CO2	1	38.32	-102.7
EF215558.1	ORC12	1	43.55	-124.22	EF215616.1	CO3	2	38.32	-102.7
EF215559.1	ORC13	3	43.55	-124.22	EF215617.1	CO4	2	38.32	-102.7
EF215560.1	ORC14	8	43.55	-124.22	EF215618.1	CO5	14	38.32	-102.7
EF215561.1	ORC15	3	43.55	-124.22	EF215619.1	KS1	1	38.18	-98.5
EF215562.1	HUMB1	2	40.63	-124.3	EF215620.1	KS2	15	38.18	-98.5
EF215563.1	HUMB2	2	40.63	-124.3	EF215621.1	KS3	1	38.18	-98.5
EF215564.1	HUMB3	1	40.63	-124.3	EF215622.1	KS4	2	38.18	-98.5
EF215565.1	HUMB4	2	40.63	-124.3	EF215623.1	KS5	1	38.18	-98.5
EF215566.1	MONT1	2	36.74	-121.8	EF215624.1	KS6	7	38.18	-98.5
EF215567.1	MONT2	9	36.74	-121.8	EF215625.1	KS7	2	38.18	-98.5
EF215568.1	MONT3	2	36.74	-121.8	EF215626.1	KS8	2	38.18	-98.5
EF215569.1	MONT4	2	36.74	-121.8	EF215627.1	KS9	2	38.18	-98.5
EF215570.1	SANTB1	2	34.41	-119.88	EF215628.1	KS10	2	38.18	-98.5
EF215571.1	SANTB2	1	34.41	-119.88	EF215629.1	KS11	2	38.18	-98.5
EF215572.1	SANTB3	1	34.41	-119.88	EF215630.1	KS12	1	38.18	-98.5
EF215573.1	SANTB4	10	34.41	-119.88	EF215631.1	KS13	1	38.18	-98.5
EF215574.1	SANTB5	2	34.41	-119.88	EF215632.1	KS14	9	38.18	-98.5
EF215575.1	SCA1	2	36.6	-117.12	EF215633.1	KS15	1	38.18	-98.5
EF215576.1	SCA2	1	36.6	-117.12	EF215634.1	OK1	2	36.73	-98.13
EF215577.1	SCA3	1	36.6	-117.12	EF215635.1	OK2	1	36.73	-98.13
EF215578.1	SCA4	2	36.6	-117.12	EF215636.1	OK3	7	36.73	-98.13
EF215579.1	SCA5	2	36.6	-117.12	EF215637.1	OK4	1	36.73	-98.13
EF215580.1	SCA6	2	36.6	-117.12	EF215638.1	OK5	2	36.73	-98.13
EF215581.1	SCA7	2	36.6	-117.12	EF215639.1	OK6	1	36.73	-98.13
EF215582.1	SCA8	1	36.6	-117.12	EF215640.1	OK7	2	36.73	-98.13
EF215583.1	SCA9	1	36.6	-117.12	EF215641.1	OK8	12	36.73	-98.13
EF215584.1	SCA10	2	36.6	-117.12	EF215642.1	TX1	2	26.98	-97.38
EF215585.1	SCA11	1	36.6	-117.12	EF215643.1	TX2	2	26.98	-97.38
EF215586.1	SCA12	2	36.6	-117.12	EF215644.1	TX3	2	26.98	-97.38

Table S3.6 continued

Genbank accession	ID	h	Lat	Long
EF215645.1	TX4	1	26.98	-97.38
EF215646.1	TX5	1	26.98	-97.38
EF215647.1	TX6	1	26.98	-97.38
EF215648.1	TX7	16	26.98	-97.38
EF215649.1	TX8	15	26.98	-97.38
EF215588.1	SCA14	2	36.6	-117.12
EF215589.1	SCA15	2	36.6	-117.12
EF215590.1	ORSL1	2	42.63	-120.23
EF215650.1	TX9	2	26.98	-97.38
EF215651.1	TX10	2	26.98	-97.38
EF215652.1	LA1	1	29.77	-93.46
EF215653.1	LA2	1	29.77	-93.46
EF215654.1	LA3	1	29.77	-93.46
EF215655.1	LA4	1	29.77	-93.46
EF215656.1	LA5	1	29.77	-93.46
EF215657.1	FL1	2	26.67	-82.25
EF215658.1	FL2	2	26.67	-82.25
EF215659.1	FL3	1	30.29	-86.73
EF215660.1	FL4	2	30.29	-86.73
EF215661.1	FL5	2	30.29	-86.73
EF215662.1	FL6	2	30.29	-86.73
EF215663.1	FL7	2	30.29	-86.73
EF215664.1	FL8	1	30.29	-86.73
EF215665.1	FL9	1	29.66	-84.95
EF215666.1	FL10	2	29.66	-84.95
EF215667.1	FL11	2	30.2	-86.08
EF215668.1	FL12	2	30.25	-87.37
EF215669.1	FL13	2	30.23	-87.5
EF215670.1	FL14	2	30.23	-87.5
EF215671.1	FL15	2	30.23	-87.5
EF215689.1	HAIT61	12	18.59	-72.01
EF215690.1	HAIT62	2	18.59	-72.01
EF215672.1	BERM63	2	32.29	-64.75
EF215673.1	BERM64	2	32.29	-64.75
EF215674.1	BAHA65	18	21.47	-71.13
EF215675.1	BAHA66	18	21.8	-71.77
EF215676.1	PR1	19	17.97	-67.19
EF215677.1	PR2	19	17.97	-67.19
EF215678.1	PR3	19	17.97	-67.19
EF215679.1	PR4	19	17.97	-67.19
EF215680.1	PR5	19	17.97	-67.19
EF215681.1	PR6	19	17.97	-67.19
EF215682.1	PR7	19	17.97	-67.19
EF215683.1	PR8	18	17.97	-67.19
EF215684.1	PR56	7	17.97	-67.19
EF215685.1	PR57	19	17.97	-67.19
EF215686.1	PR58	2	17.97	-67.19
EF215687.1	PR59	18	17.97	-67.19
EF215688.1	PR60	2	17.97	-67.19
EF215691.1	YUC68	22	21.35	-89.07
EF215692.1	PE1	18	-12.52	-76.73
EF215693.1	PE2	18	-13.7	-76.22
EF215694.1	PE3	25	-13.7	-76.22
EF215695.1	PE4	18	-11.1	-77.62
EF215696.1	PE5	23	-5.57	-80.85
EF215697.1	PE6	18	-17.1	-71.9
EF215688.1	PR60	2	17.97	-67.19
EF215691.1	YUC68	22	21.35	-89.07

Genbank accession	ID	h	Lat	Long
EF215692.1	PE1	18	-12.52	-76.73
EF215693.1	PE2	18	-13.7	-76.22
EF215695.1	PE4	18	-11.1	-77.62
EF215696.1	PE5	23	-5.57	-80.85
EF215697.1	PE6	18	-17.1	-71.9
EF215687.1	PR59	18	17.97	-67.19
EF215694.1	PE3	25	-13.7	-76.22

(A)



(B)

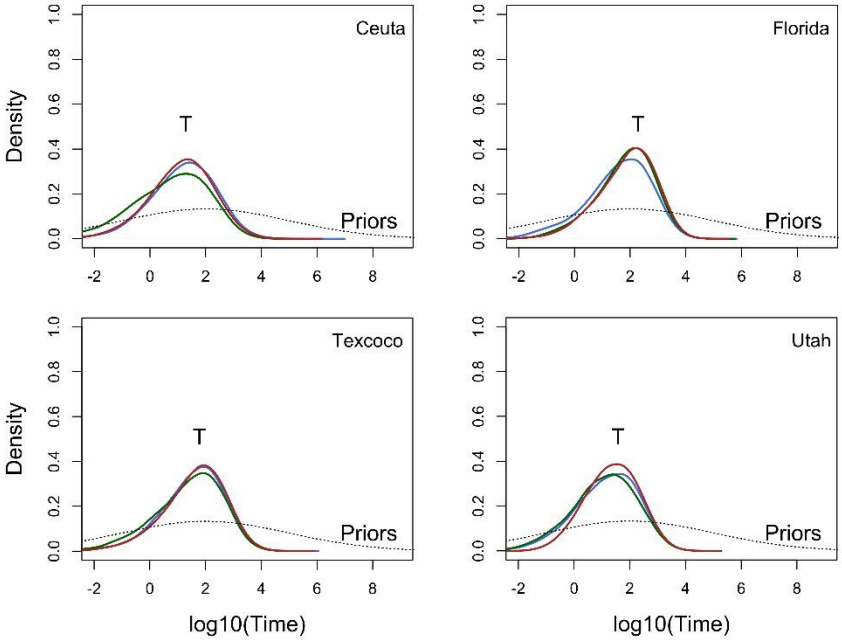


Figure S3.1 Snowy plover *Charadrius nivosus nivosus* subspecies current (N_0) and ancestral (N_1) effective population size (A) and time since population bottleneck (T) (B). Posterior distributions from three independent runs (solid lines) were calculated in Msvar (Beaumont 1999, Storz and Beaumont 2002), dashed lines represent prior distributions. Time of bottleneck and effective population size are in $\log_{10}$ scale.

Commentary Post Chapter 3

In this chapter I comprehensively reassessed current subspecies delineations and predicted the origin of low diversity in snowy plovers using demographic methods. I concluded that current subspecies are genetically supported and all three have gone through a strong population bottleneck. In addition, sub-structuring within *C. n. nivosus* indicates the Floridian snowy plover population may represent a unique population segment.

In addition to thoroughly assessing the demographic history of each deme independently, I also performed ABC methods to determine the divergence and migration between demes. However, there were few differences found between the models, and parameter prior distributions could not be distinguished from those of the priors. Therefore, the analysis was discontinued. My failure to characterise the divergence time and migration estimations within the *nivosus* subspecies could be due to a low resolving power of these markers for modelling complex demographic histories and an insufficient number of summary statistics available to use (Robert *et al.* 2011, Chu *et al.* 2013, Sunnåaker *et al.* 2013), or simply because these demes are not differentiated enough to model as separate populations.

I found contrasting signals of population structure from Z-linked loci which most likely reflects the lack of power in these few loci (n= 65). As demonstrated from this chapter itself, the detection of fine scale genetic structuring improves when increasing the number of markers. Therefore, with so few Z-linked clusters identified, it is likely that the conflicting differentiation patterns are the result of low power rather than being biologically relevant.

In addition to the analyses presented with this Chapter, I also investigated the influence polyandry can have on the genetic diversity of different genomic regions. The snowy plover is a well-studied classically polyandrous shorebird with a strongly male biased adult sex ratio, estimated at 0.63 (proportion of males in the population, Eberhart-Phillips *et al.* 2017). With more males in the population, the effective population size of the Z chromosome is greater than under equal sex ratios (Figure C3.1). This is because an equal sex ratio corresponds to four autosomes, three Z chromosomes and one W chromosome, resulting in a relative effective population size of the different genomic regions of 1:0.75:0.25. The effect of sexual selection, via skewed mating opportunities (Andersson 1994) and biased sex ratios (Liker *et al.* 2013), on genetic diversity is well documented in ZW and XY systems (reviewed by Irwin 2018, Wilson Sayres 2018). However, the examination of sexual selection and relative chromosomal diversity has traditionally focused on polygyny rather than polyandry (e.g. Charlesworth 2001, Corl and Ellegren 2012, Harrison *et al.* 2015). Under polygyny, the expected Z chromosome diversity is predicted to be lower than 0.75 due to fewer males contributing to the gene pool, whereas, in polyandry the reverse is expected (Charlesworth 2001). However, to my knowledge this only been assessed in one classically polyandrous species, the red-necked phalarope (*Phalaropus lobatus*). However, this species has such a low frequency of polyandry that it was considered monogamous in a pairwise mating system comparison of Z chromosome and autosome diversity (Corl and Ellegren 2012).

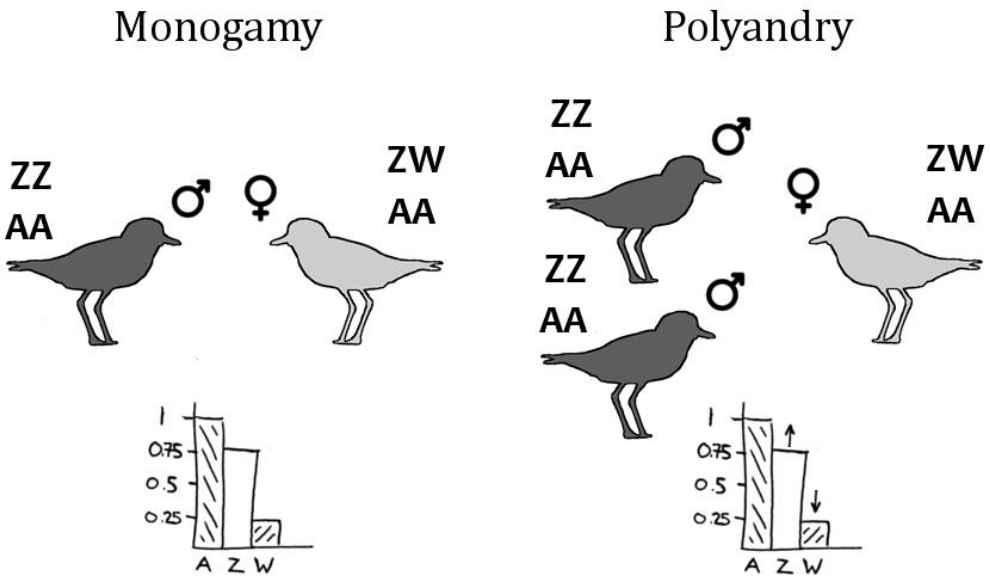


Figure C3.1. Schematic diagram illustrating how male biased sex ratio (associated with polyandry) can increase the effective population size of the Z chromosome compared to in equal sex ratios (often associated with monogamy). A = autosome, Z = Z chromosome, W = W chromosome/ mtDNA.

To extend my investigation into the genetic consequences of mating systems I used the snowy plover ddRAD loci to compare the genetic diversity (Watterson's theta (θ_W) and nucleotide diversity (π)) between the Z chromosome and autosomes for each snowy plover population. Following methods explained in Irwin (2018), I used diversity as a proxy for effective population size, and I corrected for male mutation bias (Bartosch-Härlid *et al.* 2003) by multiplying the observed autosomal diversity by 1.1 (Irwin 2018). To calculate relative Z chromosome to autosome (N_Z/N_A) diversity ratios I divided the Z chromosome diversity by the autosomal diversity. θ_W and π were calculated with the *PopGenome* package (Pfeifer *et al.* 2014) in R statistical software and I included sequence data from males only. To prevent biased diversity estimates, θ_W and π were calculated from all quality filtered ddRAD clusters, prior to excluding those that were non-polymorphic.

Table C3.1. Genetic diversity ratio of Z-linked loci compared to autosomal loci (N_Z/N_A) of the snowy plover (*Charadrius nivosus*) subspecies and sampled populations. Genetic diversity is estimated by per site Watterson's theta (θ_W) and nucleotide diversity (π).

		nivosus		***tenuirostris***	***occidentalis***
	Ceuta (4)	Utah (3)	Florida (5)	(5)	(6)
N_Z/N_A π	1.01	0.92	0.83	0.84	1.15
N_Z/N_A θ_W	0.95	0.92	0.87	0.75	1.10

With the exception of *tenuirostris*, all populations had π and θ_W N_Z/N_A ratios above 0.75, and in Ceuta (*nivosus*) and *occidentalis* this rises above 1 (Table C3.1). The diversity of the Z chromosome in snowy plovers is, therefore, well above expectations under neutrality (and equal sex ratios). This provides further evidence that mating systems, specifically adult sex ratios, can have substantial effect on the genetic diversity even at the chromosome level. Snowy plovers represent a sequentially (or serially) polyandrous species, therefore, to fully explore the consequences of mating systems, it is important to understand the implications of simultaneous polyandry. Simultaneous polyandry represents the extreme extent of classical polyandry in birds and is defined by a female being able to lay several clutches of young, fathered by different males, within a short space of time (Graul *et al.* 1977).

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Appendix B: Statement of Authorship

This declaration concerns the article entitled:									
Genomic signatures of classical polyandry in *Actophilornis* jacanas									
Publication status (tick one)									
draft manuscript	**X**	**Submitted**		**In review**		**Accepted**		**Published**	
Publication details (reference)									
Candidate's contribution to the paper (detailed, and also given as a percentage).	J.D.J formulated the ideas for the project, and designed the study and methodologies with assistance from M.W.B and T.S. J.D.J collected samples in South Africa and Madagascar and assisted with grant funding application for additional samples. J.D.J. conducted bioinformatics processing for RAD data with assistance from co-authors. J.D.J wrote the first draft of the manuscript. The manuscript has been improved further by co-authors. All figures were created by J.D.J. **J.D.J. contribution 90%**								
Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.								
Signed					**Date**				

Commentary Pre Chapter 4

In Chapter 4, I extend the findings from Chapters 2 and 3 to examine the consequences of simultaneous classical polyandry in two jacana species (Family: Jacanidae) belonging to the Genus, *Actophilornis.* Simultaneous classical polyandry is the most common breeding tactic of jacana species (Family: Jacanidae, Jenni 1996) and the most extreme form of polyandry (Graul *et al.* 1977). This breeding tactic is comparatively rarer than sequential classical polyandry, in which after one clutch is laid, a female moves to breed with a different male. Species exhibiting sequential classical polyandry include snowy plovers (*Charadrius nivosus*, Warriner *et al.* 1986, Eberhart-Phillips *et al.* 2017) and spotted sandpipers (*Actitis macularius*, Maxson and Oring 1980).

Here I investigate the genomic consequences of simultaneous classical polyandry at three scales, macro-evolutionary, micro-evolutionary, and within the chromosomes using restriction site associated DNA sequencing (Miller *et al.* 2007, Baird *et al.* 2008) and mitochondrial fragment sequencing.

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4

Genomic signatures of classical polyandry in *Actophilornis* jacanas

Josephine D'Urban Jackson, Mike R. Miller, Sama Zefania, Jeffery Peters, Graeme Cumming, Aaron Caron, Tamás Székely, and Michael W. Bruford.

African jacana by Amy Schwartz

"As role models for human beings, African Jacanas probably get a poor rating." ***W. Tarboton****. The Bird that Walks on Water*

Author contributions

J.D.J. Idea conception, sample collection, DNA extraction, conducted analysis, manuscript preparation
S.Z. sample collection
M.R.M. genomic sequencing facilities, advice on methodologies
J.P., G.C, and A.C. sample collection
T.S. idea development, manuscript improvement
M.W.B idea development, advice on methodologies, manuscript improvement

Genomic signatures of classical polyandry in *Actophilornis* jacanas

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Abstract

Jacanas (Family: Jacanidae) represent some of the most extreme examples of classical polyandry, which is defined by male only parental care and multiple mating by females. In theory, the associated traits of classical polyandry (male biased sex ratio and a skew in female mating success) have the potential to generate significant changes in genomic diversity at micro- and macro-evolutionary scales, by increasing the genetic diversity of the Z chromosome. However, empirical evidence of these changes is lacking. The Genus *Actophilornis* consists of two classically polyandrous species, the Madagascar (*Actophilornis albinucha*) and the African jacana (*Actophilornis africanus*). Using restriction site associated, and mitochondrial (mt), DNA sequencing, we examined sex specific genomic diversity and spatial genetic patterns in both *Actophilornis* species. Our study focused on the following objectives: 1) to compare genetic diversity on the Z chromosome (N_Z) and the autosomes (N_A), 2) to estimate past demographic changes in the nuclear genome, 3) to assess autosomal and sex chromosome spatial genetic structure, and finally, 4) to compare inter- and intra- specific divergence among the markers. We found extremely high relative N_Z/N_A genetic diversity in both species, and we suggest this results from highly male biased sex ratios and a skew in female reproductive success. Although, this pattern could also have arisen from population expansions, which were indicated using approximate Bayesian computations from autosomal and Z chromosome datasets. MtDNA diversity was low, supporting our hypothesis that polyandry is associated with the proliferation of few, widespread female lineages. The African jacana samples comprised two genetic clusters, dividing The Gambia from the southern African populations and we found no evidence of sex-biased dispersal with nuclear loci. The Madagascar jacana was genetically panmictic. Supporting our prediction that high Z chromosome diversity reduces the opportunity for genetic drift on this chromosome, autosomal intra- and inter-specific divergence was greater than on the Z chromosome. These results highlight the need for further empirical and theoretical work to disentangle the effects of classical polyandry at micro- and macro-evolutionary scales.

Keywords: classical polyandry, genetic diversity, jacana, Z chromosome, Fast-Z, demography, RADseq, Africa, Madagascar

Introduction

Mating systems can have a profound influence on biodiversity, from shaping genomic variation (e.g. Corl and Ellegren 2012), to modulating gene-flow (e.g. Shaw *et al.* 2018), and driving speciation (e.g. Janicke *et al.* 2018). Our understanding of mating systems is fundamental to the interpretation of evolutionary patterns and can inform conservation management (Holman and Kokko 2013). Classical polyandry, defined by minimal or no female parental care (sex-role reversal), and high female-female competition for mate access (Trivers 1972, Williams 1975, Emlen and Oring 1977, Andersson 1994) is the rarest and least understood mating system (Lack 1968, Graul *et al.* 1977, Ligon 1999, Eens and Pinxten 2000, Andersson 2005).

Although examples of classical polyandry can be found across the animal kingdom, the majority are found within species of birds and fish (reviewed by Andersson 2005, Eens and Pinxten 2000). Shorebirds have a disproportionately large number of classically polyandrous species, including in plovers (Family: Charadriidae) and sandpipers (Family: Scolopacidae) (Andersson 1994, Ligon 1999). However, one of the most extreme forms of classical polyandry, simultaneous classical polyandry, has been described or suggested in seven of the eight jacana (Family: Jacanidae) species (Jenni 1996, Eens and Pinxten 2000). Unlike sequential polyandry where females breed with different males one after the other, simultaneous polyandry involves several male territories encompassed within a single female territory, and the simultaneous mating of different males within a short time frame (Graul *et al.* 1977).

Jacanas, also known as "lilytrotters", inhabit the world's tropical and subtropical freshwater wetlands. Their movements are largely determined by rainfall and seasonally abundant habitat (Tarboton 1995). Females in polyandrous jacana species experience high variation in reproductive success and stronger sexual selection pressure than males (Emlen *et al.* 2004, Kvarnemo and Simmons 2013). Female African jacanas (*Actophilornis africanus*) have been known to lay up to 30 clutches in a breeding season with six different male partners (Tarboton 1992a, b 1996), however, the number of male mates per female can vary depending on environmental conditions (Tarboton 1995). Polyandrous jacana species have heavily male biased adult sex ratios, often two males per female (Jenni and Collier 1972, Tarboton 1992a, Butchart 2000, Emlen *et al.* 2004). *Actophilornis* jacanas (*A. africanus* and *Actophilornis albinucha*) are endemic to sub-Saharan Africa and Madagascar respectively. Although the mating system of the African jacana has been well studied (Tarboton 1992a, b, 1993, 1995, 1996, Bonkewitzz 1997), the Madagascar jacana has been ignored until recently (Chapter 5) and has been inferred to exhibit classical polyandry based on sexual size dimorphism, and phenotypic and phylogenetic similarity with the African jacana (Chapter 5).

Sex biases in reproductive success and the sex ratio of breeding adults in a population can have considerable influence on the genomic diversity and population divergence of the autosomes and sex chromosomes (reviewed by Ellegren 2009, Irwin 2018, Wilson Sayres 2018). However, so far studies have focused on male, rather than female, variation in reproductive success (Charlesworth 2001, Bartosch-Härlid *et al.* 2003, Vicoso and Charlesworth 2009, Corl and Ellegren 2012, Huang and Rabosky 2015, Wilkinson *et al.* 2015,

Wright *et al.* 2015). Therefore, our understanding of how high female reproductive skew and male biased adult sex ratios, which are commonly associated with classically polyandrous species (Emlen and Oring 1977, Andersson 1994, 2005, Kvarnemo and Simmons 2013, Liker *et al.* 2013), influence genomic diversity and intra- and inter-specific divergence remains limited.

Effective population size

Variance in mating success can lead to changes in the expected effective population size (N_e) of males and females (Caballero 1995), which can have a knock-on effect on the N_e of the sex chromosomes (Charlesworth *et al.* 1987, reviewed by Wilson Sayres 2018). In female heterogametic systems (ZW), the mitochondrial (mt)DNA and the W chromosome are maternally inherited, whereas, the Z chromosome is present in the male germline twice as much as in females. Therefore, we are able to assess the sex specific consequences of mating systems by comparing the relative diversity of the autosomes, the Z chromosome and mtDNA (Charlesworth 2001).

Sexual selection is associated with high variation in reproductive success (Andersson 1994) and skewed sex ratios (Emlen and Oring 1977, Liker et al. 2013). Consequently, departures from the expected genetic diversity ratios of 1:0.75:0.25(autosomes: Z chromosome: W chromosome/mtDNA) has been associated with sexual selection and mating systems theoretically (Charlesworth *et al.* 1987, Charlesworth 2001) and empirically for a number of wild bird populations (Hogner *et al.* 2012, Corl and Ellegren 2012, Verkuil *et al.* 2014, Huang and Rabosky 2015, Oyler-McCance *et al.* 2015, Irwin 2018). For example, in three pairs of contrasting shorebird species (one monogamous, one polygynous), Corl and Ellegren (2012) described a reduction of Z chromosome to autosome diversity (N_Z/N_A herein) below 0.75 in polygynous species. Corl and Ellegren (2012) predicted this was directly related to the degree of polygyny, which reduced the effective population size of the Z chromosome compared to under equal mating opportunities (monogamy). This study did include a classically polyandrous species, the red necked phalarope (*Phalaropus lobatus*), however, due to the low frequency of polyandry in this species (Whitfield 1990), it was considered monogamous (Corl and Ellegren 2012). Reduced N_Z/N_A ratio has also been described in sage grouse (genus *Centrocercus*), which defend leks of prospective females during the breeding season (Oyler-McCance *et al.* 2015). In another lek breeding shorebird, the Ruff, *Philomachus pugnax*, Verkuil *et al.* (2014) found that lifetime reproductive skew could be detected through reduced variation in the autosomes compared to the maternally inherited mtDNA. In contrast, using genomic methods, Huang and Rabosky (2015) found that bird species under intense sexual selection pressure (measured by plumage dichromatism), had higher N_Z/N_A ratios than their monochromatic equivalents, which they suggested was due to a reduction in present day sexual selection pressure. In classically polyandrous ZW species, which is equivalent to polygyny in XY species, we predict the N_Z/N_A ratio > 0.75 due to a higher effective population size of males (ZZ) compared to females (ZW) (Charlesworth 2001, Vicoso and Charlesworth 2009, Evans and Charlesworth 2013, Wright and Mank 2013). Under the same assumptions of small female effective population size in classically polyandrous species, we would expect low mitochondrial diversity, reflecting the proliferation of few female lineages.

Demography

Increases in the N_Z/N_A ratio can, however, also be the result of strong selection, the presence of stable mutations, or rapid demographic expansions (Pool and Nielsen 2007, Ellegren 2009). Studies of XY systems have found that the low diversity of the sex chromosomes compared to autosomes made them more susceptible to stochastic change and selection (Pool and Nielsen 2007). For example, sex chromosomes were more vulnerable to population bottlenecks, however, they were also able to recover faster post-bottleneck compared to the autosomes (Handley *et al.* 2006, Pool and Nielsen 2007). Despite the strong influence that demography has on the N_Z/N_A ratio, comparative demographic analysis of the Z chromosome and autosomes is rarely conducted, and disentangling the effects of evolutionary processes from those resulting from demographic processes is challenging (Van Belleghem *et al.* 2018). In this study, we hypothesise that classical polyandry is associated with detectable signals of population expansions on the Z chromosome and the autosomes in both *Actophilornis* jacanas.

Spatial genetic patterns

Spatial genetic patterns are often interpreted using past or present environmental or anthropogenic variables (e.g. Orsini *et al.* 2013, Wang *et al.* 2013, Thomassen *et al.* 2018). Nevertheless, social behavioural drivers of genetic population structure, for example mating systems, are well documented (Storz *et al.* 2001, Ross 2001, Francisco *et al.* 2007, Küpper *et al.* 2012, Ribeiro *et al.* 2012, Carroll *et al.* 2015, van Dijk *et al.* 2015, Parreira and Chikhi 2015, Morinha *et al.* 2017, Shaw *et al.* 2018, Uy *et al.* 2018). However, there is currently little evidence of how classical polyandry can induce sex-biased dispersal and consequently sex-specific population genetic patterns (D'Urban Jackson *et al.* 2017). Possible mechanisms for sex-biased dispersal in classically polyandrous species include the following: Female territory acquisition and defence is essential for breeding success in New World jacanas (Emlen *et al.* 2004), therefore, territoriality may promote greater site fidelity of females than in males. This hypothesis can be generally considered as an "asset-protection-principal", whereby dispersal is restricted to ensure the territory holder gains the benefits of their asset (Clarke 1994, Harts *et al.* 2015). Alternatively, Greenwood (1980) predicted that in resource defence systems, dispersal will be biased towards the territory acquiring sex. It is unclear how the defence of resources will influence sex-biased gene-flow in *Actophilornis* jacanas because both sexes defend territories, with male territories located within larger, female territories (Tarboton 1995). Female sex-biased dispersal in jacanas may also be promoted by competitive avoidance with kin (Perrin and Mazalov 2000, Waser *et al.* 2013, Brom *et al.* 2016) or with other females (Dobson 1982). Furthermore, as the limiting sex, female biased inbreeding avoidance strategies may promote their dispersal (Pusey 1987, Perrin and Mazalov 2000) or the pressure to find multiple breeding partners, also known as the "dispersal-to-mate" hypothesis (D'Urban Jackson *et al.* 2017, Végvári *et al.* 2018).

Speciation and population divergence

The role of sex chromosomes in speciation and population divergence has recently received increased attention (Mank 2012, Meisel and Connallon 2013, Dean *et al.* 2015, Irwin 2018, Moran *et al.* 2018, Van Belleghem *et al.* 2018). The Z chromosome is thought to play a disproportionately large role in population divergence and speciation compared to the autosomes (Meisel and Connallon 2013, Irwin 2018, Presgraves 2018). This is hypothesised

to be due to two main reasons: male mutation bias (reviewed by Ellegren 2007) and 'Fast-Z' evolution (reviewed by Meisel and Connallon 2013, Presgraves 2018). During sperm production, a greater number of cell divisions occur compared to egg production (Miyata *et al.* 1987). Mutations are the result of errors during replication, therefore, more mutations are expected in the male line compared to the female line, and this is termed male mutation bias (Miyata *et al.* 1987). In birds, sexual selection in males is correlated with mutation rate, however, the effect of female mating success variation remains untested (Bartosch-Härlid *et al.* 2003). Fast-Z (or X) is a well-established theory which explains that because the chromosome is hemizygous in one sex, selection on recessive beneficial alleles in the hemizygous sex can act faster on the Z chromosome compared to the autosomes, due to the lack of masking by a second chromosome (Charlesworth *et al.* 1987). However, deleterious alleles can also become fixed on the Z chromosome due to genetic drift as a result of weaker purifying selection, which is caused by a smaller N_e on the Z (or X) chromosome compared to autosomes (Vicoso and Charlesworth 2009). The reduction in N_e is further exacerbated in polygynous ZW species due to high skew in male reproductive success (Mank *et al.* 2010, Wright *et al.* 2015). In classically polyandrous species, contrasting evolutionary processes may occur because Z chromosome diversity is elevated rather than reduced (Charlesworth 2001) as a result of male biased sex ratios and high sexual selection pressure on females (Kvarnemo and Simmons 2013). Therefore, this mating system may reduce or eliminate the signal of 'Fast-Z' evolution, leading to similar degrees of divergence on Z chromosome and autosomes, although this phenomenon has yet to be explored.

Here we use a combination of high throughput, and mtDNA, sequencing to provide the first genome-wide assessment of classical polyandry in jacanas. We take advantage of a multi-species and multi-population approach to comprehensively assess intra- and inter-specific diversity and divergence in both species of *Actophilornis* jacanas. Our work complements previous research on the genetic consequences of mating systems in ZW systems, which have so far neglected classical polyandry. In addition, this study constitutes the first population genetic study of the African and Madagascar jacanas. We test the following hypotheses: 1) male biased sex ratio and skewed female mating success associated with classical polyandry will result in N_Z/N_A ratios > 0.75, 2) demographic history modelling will indicate population expansions in both species, 3) polyandry will promote female biased dispersal and male site fidelity, leading to stronger Z-linked isolation by distance and genetic clustering compared to autosomal and mtDNA markers and, 4) higher Z chromosome diversity will reduce the opportunity for 'Fast–Z' evolution, and result in similar intra- and inter-specific divergence on the Z chromosome and the autosomes.

Methods

DNA extraction

Blood samples of African (n=230) and Madagascar jacana (n=55) stored in either 90% ethanol or queens lysis buffer (Seutin *et al.* 1991) were collected from across both species' ranges between 2008 and 2017 (Figure 4.1, Table 4.1). In addition, two African jacana tissue samples from wild Ugandan birds that died in captivity at the Zoological Society of London were extracted and sequenced for mitochondrial DNA. Madagascar and southern African jacana blood samples were collected as part of two previous studies (Chapter 5, Cumming *et al.* 2011), except for four samples from South Africa which were collected for this study. For

newly collected samples from South Africa and The Gambia, 25 µl blood was collected by puncturing the brachial vein with a 25 G sterile needle after capturing the birds in mist nets. We followed standard avian blood sampling protocols (Owen 2011) and all samples were collected and exported under permits including ethical approval from the University of Bath (Supplementary Material I.).

Genomic DNA was extracted using ammonium acetate (Bruford *et al.* 1998). To remove low quality samples unsuitable for restriction site associated DNA sequencing (RADseq) (Miller *et al.* 2007, Baird *et al.* 2008, Graham *et al.* 2015), we assessed each DNA extract by gel electrophoresis (0.7 % agarose, 100 V, 1 hr 30 mins), NanoDrop ND-1000 Spectrophotometry (Nano-Drop Technologies, Inc., Wilmington, DE, USA), and the concentration was determined using Qubit Fluorometric Calibration (QFC, Invitrogen, Carlsbad, California) with the dsDNA assay. For RADseq we prepared a final DNA volume and concentration of 50µl of 20ng/µl.

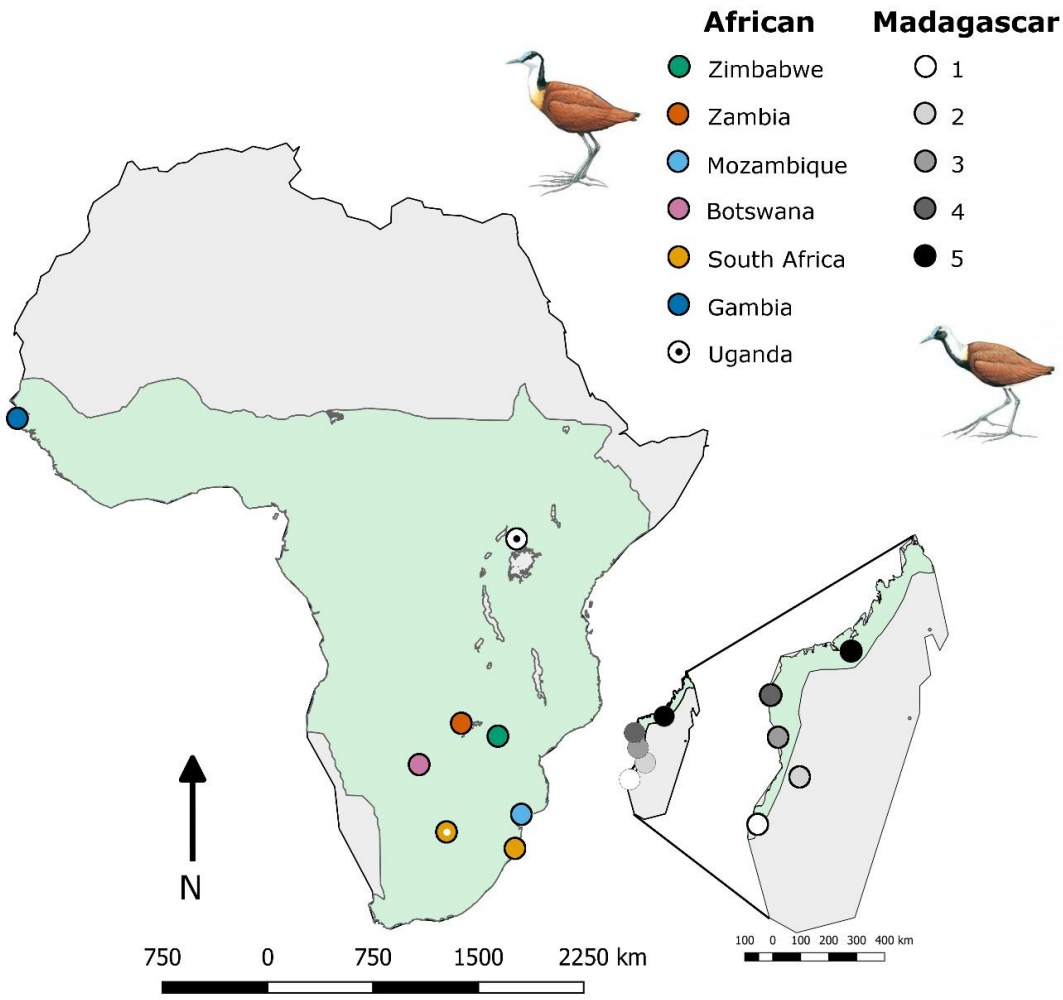

Figure 4.1. Map of sampling locations of the Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas included in this study. Green shaded areas represent the range of the two species. Madagascar jacana is endemic to Madagascar, whereas African jacana is endemic to the African mainland. South Africa samples were collected from two locations (yellow), however, just one sample was collected from the yellow circle with white dot and it was used for mtDNA only (see Table 4.1).

Sexing

Jacanas were sexed by amplifying the CHD gene, using the 2550 (5'-GTTACTGATTCGTCTACGAGA-3') and 2718 (5'-ATTGAAATGATCCAGTGCTTG-3') primer pair (Fridolfsson and Ellegren 1999) and additionally with the CHD1F (5'_TATCGTCAGTTTCCTTTTCAGGT_3') and CHD1R (5'_CCTTTTATTGATCCATCAAGCCT_3') primer pair (Lee *et al.* 2010) only if the molecular sexing conflicted with morphometric sexing predictions based on body mass (females being the significantly larger sex, Chapter 5). For both PCRs, 2 µl of *5X Green GoTaq® Flexi Buffer*, 0.8 µl of 25 mM $MgCl_2$, 0.5 µl of each primer at 10 pmol/µl, 0.2 µl of 10 µM dNTPs, 0.05 units of *GoTaq® G2 Flexi DNA Polymerase* and 1 µl of DNA (approximate concentration 5-20 ng/µl) were included in a total volume of 10 µl. The PCRs followed 94°C for 2 minutes, 40 cycles of 94°C (45 seconds), 48°C (45 seconds) and 72°C (45 seconds), and 5 minutes at 72°C. All PCR products were visualised on a 2% agarose gel run for 1 hour at 120 V, one band represented a male and two bands represented a female for both primer pairs.

Table 4.1. Samples included in the population genetic analysis of African (*Actophilornis africanus*) and Madagascar (*Actophilornis albinucha*), jacanas. African jacana (AJ) were divided into two groups reflecting genetic clusters, southern Africa and Gambia (see Figures 4.1, 4.3-4.5). Lat = degrees latitude, Long = degrees longitude, mtDNA = samples sequenced for mitochondrial ND2 fragment, RADseq = samples included for Restriction site associated DNA sequencing. F and M = number of females and males respectively.

Genetic cluster	Sampling location (code)	Lat	Long	mtDNA	RADseq (F,M)
southern Africa (AJ)	Zimbabwe (ZIM)	-17.82	30.55	37	31(12,19)
	Zambia (ZAM)	-16.65	27.01	0	4(1,3)
	Mozambique (MOZ)	-25.00	32.93	52	34(18,16)
	Botswana (BOT)	-20.50	22.80	19	13(7,6)
	Uganda** (UGA)	1.28	32.44	2	0
	South Africa* (SAF)	-28.06	32.29	5	4(3,1)
	TOTAL			**115**	**112**
Gambia (AJ)	NA	13.09	-16.76	6	**29**(9,20)
Madagascar jacana	1	-21.89	43.59	4	10(6,4)
	2	-20.34	45.11	2	8(3,5)
	3	-18.97	44.40	2	10(3,7)
	4	-17.55	44.06	3	9(4,5)
	5	-15.99	46.97	4	12(3,9)
	TOTAL			**15**	**49**

* 1 sample from an additional site: Lat: -26.58, Long: 25.59 (mtDNA only)

** no precise location data available, estimated as centre of country

mtDNA sequencing

To provide means to analyse female-biased dispersal, we amplified a 615 bp fragment of the mitochondrial NADH dehydrogenase subunit (ND2) in 115 and 15 African and Madagascar jacana, respectively (Nabholz *et al.* 2016). We used modified AsnH (5'-

GATCRAGGCCCATCTGTCTAG-3') and MetL2 (5'-TAAGCTATCGGGCCCATACCCC-3') primers (O. Haddrath pers. Comm.). In 20 µl we included 4 µl *5X Green GoTaq® Reaction Buffer*, 2 µl, $MgCl_2$ (25 Mm), 0.5 µl dNTPs (10mM), 0.5 µl of each primer (10 pmol/µl), 0.25 units of *GoTaq® G2 Flexi DNA Polymerase* and 1 µl of DNA. The thermal profile for the ND2 amplification was: 94°C for 1 minute 30 seconds, 36 cycles of 94°C for 40 seconds, 64°C for 40 seconds, 72°C for 1 minute and a final 5 minutes at 74°C.

ND2 products were sequenced by Eurofins Genomics Sequencing Services on an AB1 platform. Sequences were trimmed in Sequencher ® 5.1 (GeneCodes Corporation), and we used BioEdit v7.1.11 (Hall 1999) to align the sequences, before trimming all sequences to 615 bp. We computed diversity statistics in DNAsp v5 (Rozas *et al.* 2003, Librado and Rozas 2009). Frequencies and geographic distribution of mtDNA haplotypes were visualised with a TCS haplotype network using the minimum number of mutational steps between different haplotypes (Clement *et al.* 2002) created in PopArt (Leigh and Bryant 2015).

RADseq

RADseq libraries were prepared for 186 samples corresponding to 175 individuals with repeats. Library preparation followed the method described by Ali *et al.* (2016) using the *PstI* restriction enzyme. Libraries consisted of two sets of 96 individually barcoded samples. Individuals were sequenced with 100 bp paired-end reads with an Illumina HiSeq 2500 platform, one lane of Illumina was used per library.

Raw sequences were paired and aligned to the closest available assembled genome of *Jacana jacana* (B10K project E. Jarvis pers. comm.) in BWA using the *mem* algorithm (Li 2013). Reads were sorted and PCR duplicates were removed in SAMtools v1.1 (Li *et al.* 2009). Samples with low read counts ($<10^6$ reads) and known relatives or duplicates (selecting the highest read coverage of each set) were removed before data analysis. For genomic data with low to medium and/or variable coverage across individuals in a dataset, it is highly recommended to calculate genotype likelihoods for downstream analyses rather than calling genotypes, as this allows the incorporation of sequencing error and uncertainty (Nielsen *et al.* 2011). To do this we used the program ANGSD v0.92 (Korneliussen *et al.* 2014) and associated programs including NGStools (Fumagalli *et al.* 2014) for the majority of our analyses. We used the following filters for all analyses with ANGSD: minimum mapping quality of 20, minimum base calling quality of 20, the site must have been sequenced in at least 80% of individuals, we used the SAMtools method to calculate genotype likelihoods, a minor allele frequency cut off of 0.05, triallelic sites skipped, SNPs had to have a *p*-value less than $1e^{-6}$ for being variable to be retained, we used a uniform prior to calculate posterior probabilities, minimum depth of five and for genotype calling we used as posterior cut off of 0.95. We used the *–rf* function to restrict analyses to either autosomal or Z scaffolds (see below). All analyses directly comparing genetic data from autosomal and Z chromosomes were conducted using male only datasets.

We found no differentiation between southern African sampling sites or between Madagascar sampling sites (Figures 4.3-4.5, Supplementary Material II, Table S4.1), therefore, herein we concentrated our analyses on three genetic clusters (termed populations herein), two for the African jacana: southern Africa (which includes: Botswana, South Africa, Mozambique, Zambia, Zimbabwe) and Gambia, and one for the Madagascar jacana (as a single genetic unit).

Relatedness among individuals was estimated with NGSrelate (Korneliussen and Moltke 2015) and individuals that were identified as being highly related (r = 0.5) were removed. All remaining individuals and their populations are described in Table 4.1.

Identifying Z-linked scaffolds

To identify Z-linked SNPs for population genetic analysis we first needed to identify scaffolds of the *J. jacana* reference genome located on the sex chromosomes, for this we used a three step approach. Firstly, preliminary PCAs computed prior to filtering for loci out of Hardy Weinberg Equilibrium (HWE), split individuals into two groups for each species, corresponding to males and females (Figure S4.1). We expected that loci on the Z chromosome should be out of HWE because of heterozygote deficiency, as females are the heterogametic sex (Figure S4.2). Therefore, we extracted scaffolds which had one or more locus out of HWE (p <0.05) and used NCBI's Nucleotide BLAST (*blastn*) v2.7.1+ (Altschul *et al.* 1990, Camacho *et al.* 2009) to match these scaffolds against known Z chromosome scaffolds from the killdeer (*Charadius vociferous*) genome (Zhou *et al.* 2014). Scaffolds with E-values of 0, indicating a high match score, were selected for the final step, this included 874 scaffolds in the African jacana and 663 in the Madagascar jacana. Finally, we compared read depth at each site of the reads that mapped to the Z chromosome scaffolds between the sexes under the following assumptions: i) as females are the heterogametic sex, they will have half the read depth of males at Z-linked sites, ii) both sexes should have equal read depths at autosomal sites and iii) females should have very high read depth at W chromosome linked sites, whereas, males would have minimal depth, if any (Bidon *et al.*, 2015). For this, we used SAMtools *depth* to calculate the read depth at each site of the RAD reads (with a base calling quality and mapping quality of > 20) for 10 males and 10 females of the southern African jacana population that were selected for high coverage. For each sex, and each position, the number of reads was summed. To standardise for differences in the overall read depth between the sexes, the sum of male reads for all sites was divided by that of females. The female read depth at each site was then multiplied by this standardisation factor. Following this, the male read depth was divided by the (standardised) female read depth to produce an AD ratio at each site (Bidon *et al.* 2015). We plotted the AD ratio of all loci on a scaffold if at least one locus on that scaffold conformed to the sex linked loci expectations: >2 = Z chromosome, 1 = autosomal, 0-0.3 = W chromosome autosomal (Bidon *et al.* 2015). Although some scaffolds indicated trends consistent with their genomic region (Figure S4.3), others had high variation in their ratios across loci. To minimise error we selected only scaffolds with an average AD ratio of above 1.4 (n= 55) which represented a clearly elevated AD ratio, indicative of being on the Z chromosome (Figure S4.4).

Scaffolds that either gave inconsistent signals of being on the Z chromosome based on the BLAST search or AD ratio methods or were identified just in one species, were removed from both the Z chromosome dataset and the autosomal dataset. The remaining scaffolds were considered autosomal.

Effective population size

We compared N_e on the Z chromosome and autosomes using genetic diversity as an approximate measure (Irwin 2018). Watterson's theta (θ_w) and nucleotide diversity (π) were calculated in *realSFS* for each scaffold using a folded site frequency spectrum (SFS). Due to computational constraints, both diversity measures were calculated from a maximum

of 20 males in each population (southern Africa n=19, Gambia n=17, Madagascar n=20). In the absence of selection, the male mutation rate on the Z chromosome in birds is 1.1 times faster than the autosomes (reviewed by Irwin 2018), to control for this, autosomal diversity estimates were multiplied by 1.1, before dividing by the total number of sites for each scaffold and averaging across scaffolds. We also calculated θ_w and π over all scaffolds combined for autosomal and Z chromosome datasets independently.

We compared deviations from mutation-drift equilibrium by calculating per scaffold Tajima's D (Tajima 1989a) for Z chromosome scaffolds and autosomal scaffolds in ANGSD using a folded SFS. The significance of the deviation of Tajima's D from 0 was determined with one-tailed T-tests in R statistical software (R Development Core Team 2018). Tajima's D values were used to infer demographic trends and signals of selection. Population expansions and/or a selective sweep can result in a negative Tajima's D, whereas, a strongly positive value can result from a population bottleneck or balancing selection (Tajima 1989b).

Demography

We inferred demographic trends of the Z chromosome and the autosomes using Approximate Bayesian Computations (ABC) (Beaumont *et al.* 2002) estimated by demographic simulations created by fastsimcoal2 v2.6 (Excoffier and Foll 2011) and implemented through the ABCtoolbox framework v2 (Wegmann *et al.* 2010). ABC compares summary statistics from simulated demographic scenarios to those from observed datasets to determine the best fitting scenario and to estimate chosen parameters (e.g. current and ancestral N_e). Although this method has some limitations, it has been shown as a reliable method estimate past changes to population size (reviewed by Bertorelle *et al.* 2010). We focused this analysis on 20 males from southern Africa and Madagascar jacanas and used called genotypes from the Z chromosome (n=775) and autosomes (n= 9,231) separately with a SNP array approach. Species were analysed independently due to the potential for ascertainment bias caused by genotype calling with both species together simultaneously, which can influence ABC results (Quinto-Cortés *et al.* 2018). We tested six scenarios per species, this included a stable, bottleneck, and expansion scenario occurring at two time points (1-1,000 years ago (YA) and 1,000-100,000 YA) to represent recent versus ancient demographic patterns. Uniform prior distributions were set for each scenario (Table S4.2) and the same number of loci as in the empirical datasets were simulated (Table S4.3). We considered each locus as unlinked in both genomic regions. This was assumed because i) RAD loci are spread randomly throughout the genome (Davey and Blaxter 2010), ii) linkage is similar in the Z chromosome and autosome of birds and iii) linkage has been shown to decay within short distances in birds (100 bp, Poelstra *et al.* 2013). Including linked loci can, however, artificially narrow confidence intervals, which should be taken into account (Excoffier *et al.* 2005).

We simulated 10^5 datasets in fastsimcoal2 and computed summary statistics with arlsumstat v3.5.2.2. (Excoffier and Lischer 2010). We then measured the Euclidean distance between empirical and simulated datasets and retained the closest 1,000 simulations to our observed dataset to assess model fit. We assessed pairwise correlation between summary statistics by computing Spearman's Rho statistics, and removed any that were highly correlated before comparing models. The non-correlated statistics that were used for model comparisons were: the total number of alleles and mean heterozygosity. We calculated marginal densities and posterior probabilities for each model using the built-in general

linear post-sampling adjustment step within ABCtoolbox. Model fit was inferred for all scenarios by pairwise comparisons of the marginal densities and *p*-values (Wegmann *et al.* 2010). Models with *p*-values > 0.05 were considered viable and we selected the best fitting model based on a Bayes Factor (BF) greater than three. Generation time was set at one year for both species (W. Tarboton pers. comm.)

Genetic structure and isolation by distance

We assessed genetic structure with two methods, principle component analysis (PCA) and estimations of individual admixture proportions. To create a covariance matrix for PCAs we used the single read sampling method implemented in ANGSD. This prevents bias that can be introduced from variation in sequencing depth during standard PCA methods (Korneliussen *et al.* 2014). PCAs were plotted with R statistical software v3.5.1 (R Development Core Team 2018). We assessed individual admixture proportions with NGSadmix (Skotte *et al.* 2013) using a hierarchical approach, first including both species, then within each species, and finally, within each genetic cluster (similar to Hobbs *et al.* 2011). We tested *K* values from 2-5, repeating each *K* five times or until the replicate runs were within three likelihood units of each other (Pedersen *et al.* 2018). Admixture proportions for each *K* were merged and visualised with the program PopHelper 2.2.6. (Francis 2017).

With the absence of more detailed landscape characteristic data for either species, isolation by distance (Wright 1943) was assessed with Mantel tests in the R package, *adegenet* (Jombart 2008) with 9999 permutations. Individual genetic distance matrixes were created in ngsDist (Vieira *et al.* 2016) and Euclidean geographic distances matrixes between individuals were created in Genalix v6.5 (Peakall and Smouse 2012).

PCAs, admixture, and isolation by distance analysis were conducted within all individuals using the autosomal dataset, and within males only for comparisons between autosomal and Z chromosome SNP datasets. We additionally compared isolation by distance for adult males and females of the Madagascar jacana to infer sex-biased dispersal, as these samples were collected within a relatively short time period (9 months).

Inter- and intra-specific sequence divergence

We estimated intra- and inter-specific divergence (between southern African jacana, Gambian African jacana and Madagascar jacana) with nuclear RAD loci consensus sequences and ND2 mtDNA sequences. To calculate divergence using RAD sequence data, we selected one Z chromosome scaffold (*scaffold194*) and one autosomal scaffold (*scaffold10396*) that both contained a high density of RAD loci (visualised in Tablet v1, Milne *et al.* 2013). To extract consensus sequences of RAD loci within these two scaffolds we used a custom perl script (O. Rimmington, pers. comm., available on request). Sequences were only obtained from the high depth male samples (those used in diversity calculations) to maximise consensus sequence length. The consensus sequence was restricted to sites with a minimum read depth of eight, and any polymorphic sites were summarised by majority rule. Extracted sequences were aligned to each other and the *J. jacana* reference genome with the Clustal Omega multiple sequence alignment tool (Sievers *et al.* 2011). Alignments were trimmed across all individuals to remove blocks of missing sequence information (Ns) and to ensure the alignments of both scaffolds were of the same length for comparisons (1296 bp).

For RAD and ND2 sequences we calculated mean intra- and inter-specific distances using the Maximum Composite Likelihood method, implemented in MEGAx (Tamura *et al.* 2004, Kumar *et al.* 2018).

Results

Across 186 samples we obtained 552,793,801 sequencing reads. After filtering (see methods) 161 individuals remained (Table 4.1). From these, an average of 97 % of raw reads aligned to the *J. jacana* reference genome (min: 96 %, max: 98 %) excluding one individual of which 79 % aligned. Of the aligned reads, on average 70 % passed filtering stages (min: 56 %, max: 81 %). SNP filtering for genotype likelihoods and/or called genotypes (see methods) yielded between 300 – 775 Z chromosome SNPs, and 3,207-9,231 autosomal SNPs for analyses, depending on the genomic region selected and the individuals included (Table S4.3).

Effective population size

Genetic diversity on the autosomes and the Z chromosome was lower in the Madagascar jacana compared to the African jacana (Table 4.2, Table S4.4). Mean Tajima's D per scaffold was significantly negative for autosomes in both species (African: t=-12.68, df = 19,907, p=<0.001, Madagascar: t=-121.01, df=30,120, p=<0.001) but positive in the Z chromosome, although, this was not significant in the Madagascar jacana (African: t=4.03, df=54, p=<0.001, Madagascar: t=1.13, df=54, p=0.266) (Table 4.2, Figure S4.5). Both male mutation bias corrected and uncorrected comparisons of N_Z/ N_A were above the expected value of 0.75 under equal sex ratios and mating opportunities for males and females (Table 4.3). Across all measures, the Madagascar jacana had higher N_Z/ N_A ratios than the African jacana (Table 4.3).

Table 4.2. Genetic diversity of Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas on the Z chromosome and autosomes. Watterson's theta (θ_w) and nucleotide diversity (π) presented here was calculated overall, not per scaffold, and is uncorrected for male mutation bias. Tajima's D (standard deviation) is averaged across scaffolds. * indicates Tajima's D significance from zero $p < 0.001$

		$\boldsymbol{\theta_w}$	$\boldsymbol{\pi}$	**Tajima's D**
Madagascar	Z chromosome	0.002	0.002	0.34(0.39)
	Autosomal	0.002	0.001	-0.60(0.75)*
African (southern)	Z chromosome	0.004	0.005	0.20(1.85)*
	Autosomal	0.005	0.005	-0.07(0.66)*

Table 4.3. Relative genetic diversity of Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas Z chromosome (N_Z) compared to autosomes (N_A), (N_Z/ N_A) measured by Watterson's theta (θ_w) and nucleotide diversity (π). Columns 2 and 3 are corrected for male mutation bias.

	$\boldsymbol{\theta_w}$	$\boldsymbol{\pi}$	**uncorrected overall $\boldsymbol{\theta_w}$**	**uncorrected overall $\boldsymbol{\pi}$**
Madagascar	1.67	2.20	1.26	1.52
African (southern)	1.30	1.44	0.96	0.97

Demography

ABCs predicted population expansions between 1000-100,000 years ago in both species, and both genomic regions (Z chromosome and autosome), with Bayes factors >3 (Table S4.5, Figure 4.2). Estimations of current and ancestral N_e indicated similar degrees of expansion in the two genomic regions (Figure 4.2), however, the current N_e of the Madagascar jacana was estimated at approximately half that of the African jacana (Table S4.6). The time of the expansion was similar across region and species, and was predicted to have occurred between 33,835 YA – 71,146 YA. However, the posterior distribution of this variable was similar to the prior distribution (Figure S4.6) and there was no clear peak of the posterior distributions either species or genomic region (Figure 4.2). Furthermore, confidence intervals for all parameter estimations were very wide (Table S4.6).

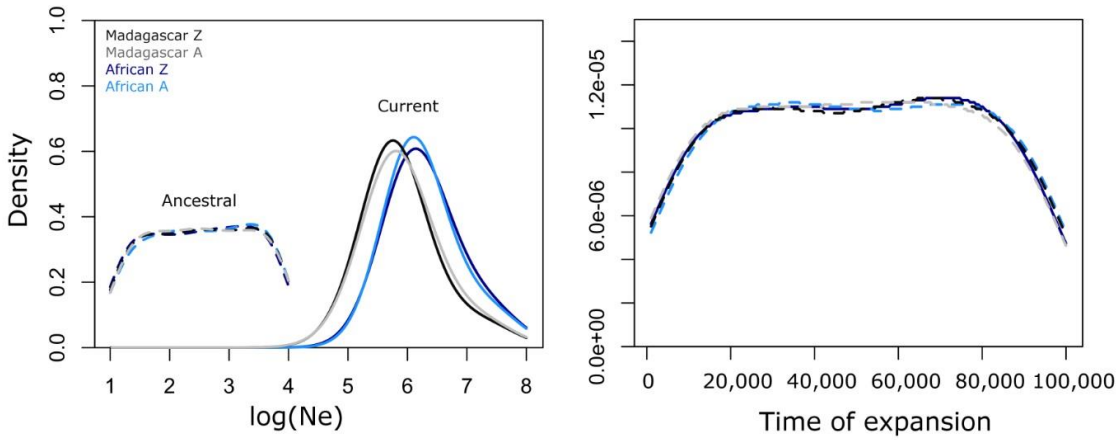

Figure 4.2. Density distributions of current (solid lines) and ancestral (dashed lines) effective population size (N_e, left) for Madagascar (grey, *Actophilornis albinucha*) and African (blue, *Actophilornis africanus*) jacanas and the time of expansion (right) estimated by approximate Bayesian computations using ABCtoolbox (Wegmann *et al.* 2010). ABCs were performed separately for Z chromosome loci (Z) and autosomal loci (A).

Genetic structure and isolation by distance

African and Madagascar jacana were clearly separated in PCA and admixture analyses (Figures 4.3, 4.4A), however, the variance explained by the clustering of each species was very low (Figure 4.4A, PC1=1.96%). Within African jacanas, two clusters were identified representing samples from The Gambia in one and the remaining southern African samples in the other (Figures 4.3 *K*=3&4, 4.4B, 4.5). Admixture of the Gambian cluster was distributed evenly within the southern populations (Figure 4.3 *K*=3&4) and there were no substantial differences between autosomal and Z chromosome admixture proportions (Figure 4.5). In the African jacana we found isolation by distance with autosomal and Z-linked loci, when including all populations (Figure 4.6). Separately within the southern populations, we found weak isolation by distance when including both males and females for autosomal loci (Figure 4.7), but not with male only comparisons of autosomal, nor of Z-linked loci (Figure S4.8A).

We found no spatial pattern of admixture across the range of the Madagascar jacana (Figures 4.3 and 4.5) and the PCA plot indicated genetic homogeneity (Figure 4.4C). We

found no signal of isolation by distance with autosomal nor Z-linked loci in the Madagascar jacana (Figure S4.8B), nor when analysing adult males and females (Figure S4.9).

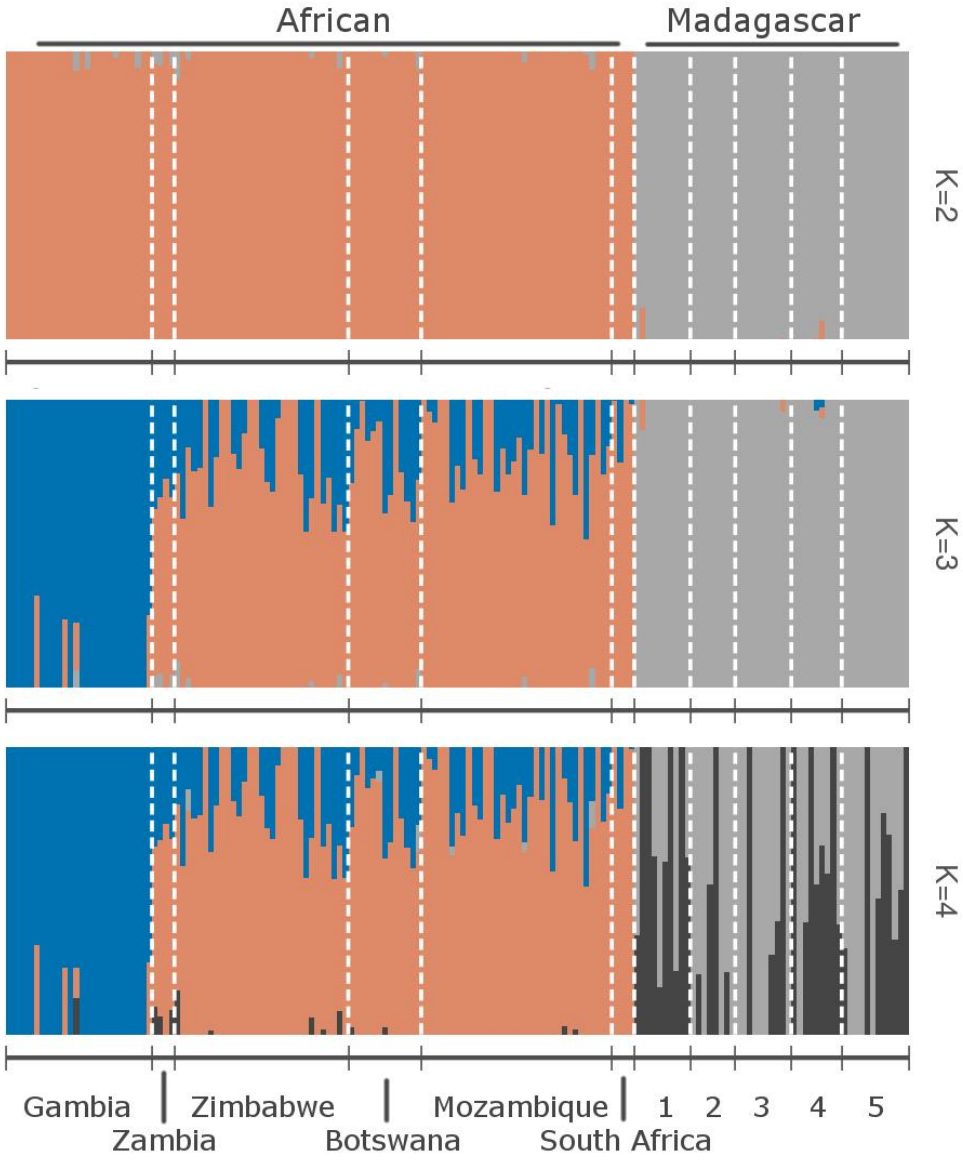

Figure 4.3. Admixture proportions of Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas for *K*=2-4 estimated by NGSadmix (Skotte *et al.* 2013) using autosomal loci. Males and females were included. For geographic positions of sampling locations see Figure 4.1 and Table 4.1.

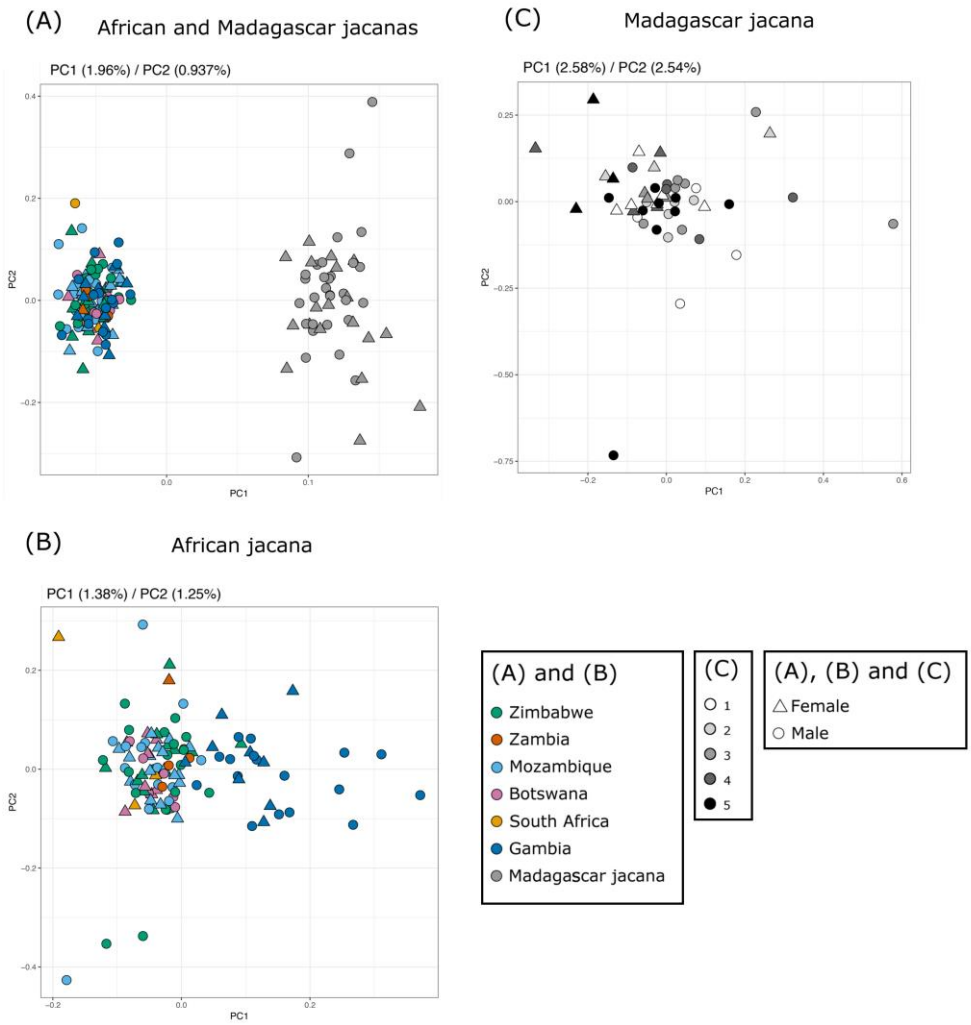

Figure 4.4. Principle component analysis (PC 1 and 2) of African (*Actophilornis africanus*, A and B) and Madagascar (*Actophilornis albinucha*, A and C) jacanas using autosomal loci. Females = triangles, Males = circles.

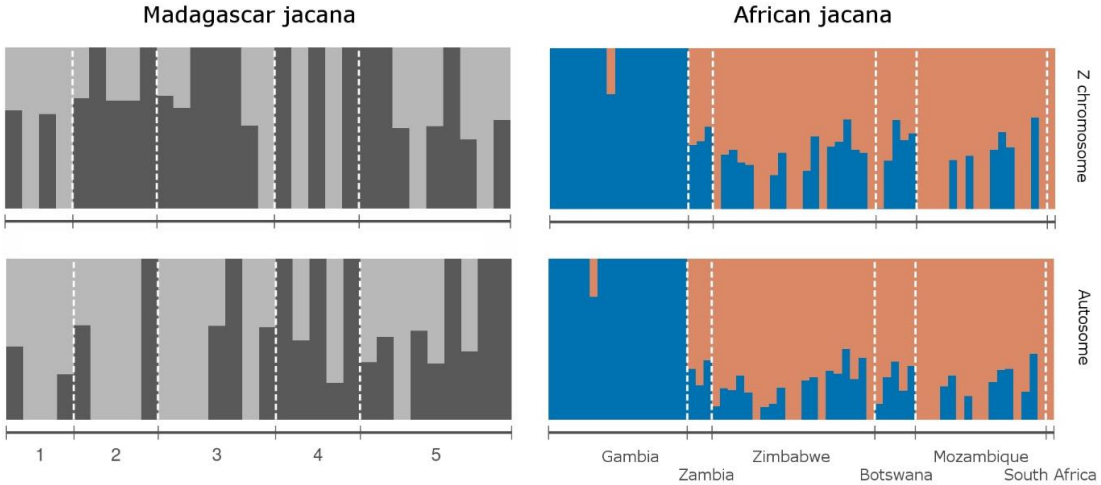

Figure 4.5. Admixture proportions of African (*Actophilornis africanus*, right) and Madagascar jacana (*Actophilornis albinucha* left) at *K*=2 using Z chromosome (upper) and autosomal (lower) loci, estimated by NGSadmix (Skotte *et al.* 2013). Only males were included in this analysis.

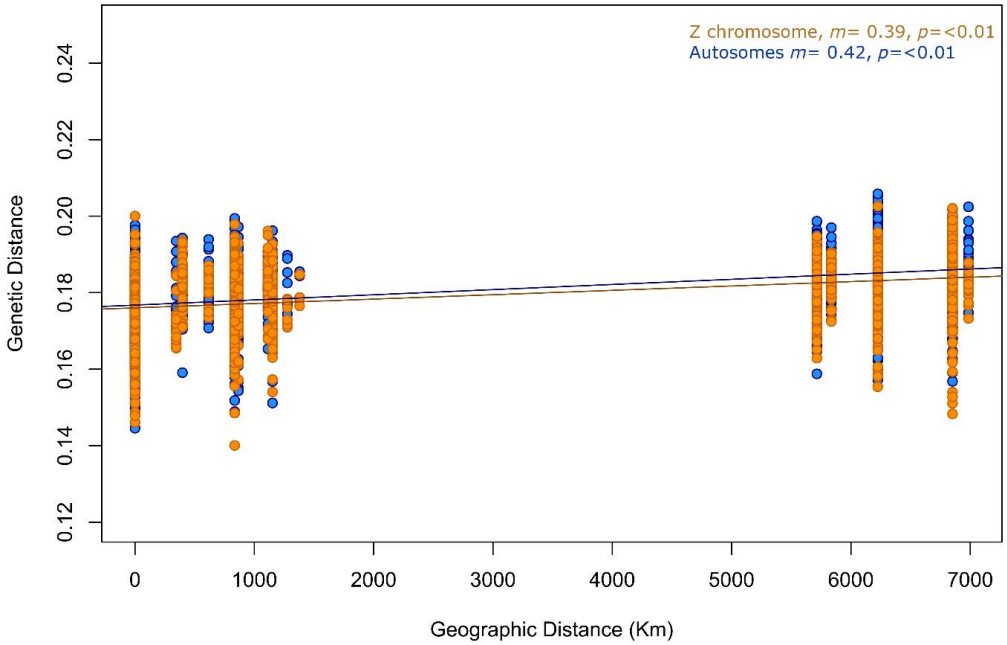

Figure 4.6. Individual pairwise isolation by distance of the African jacana (*Actophilornis africanus*) including all sampling sites for autosomal (blue) and Z chromosome (orange) loci. Only males were included in this analysis and pairwise distances were calculated using NgsDist (Vieira *et al.* 2016). Mantel test performed with 9999 permutations, statistic (*m*) and significance is shown.

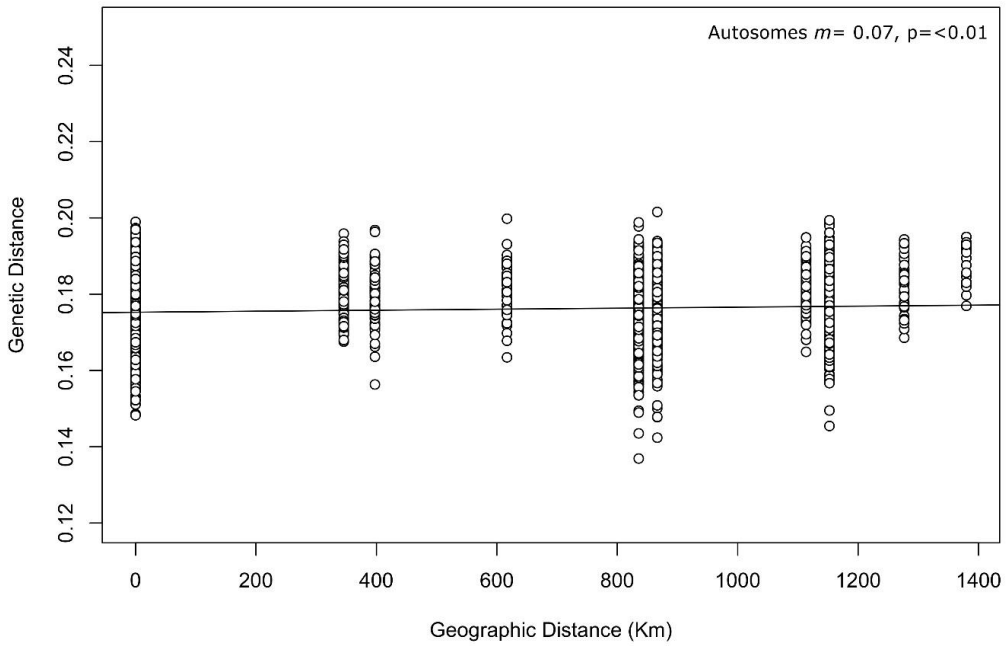

Figure 4.7. Individual pairwise isolation by distance of Africa jacana (*Actophilornis africanus*) populations from southern Africa using autosomal loci. Males and females were included and pairwise distances were calculated using NgsDist (Vieira *et al.* 2016). Mantel test performed with 9999 permutations, statistic (*m*) and significance is shown.

mtDNA

We identified 10 haplotypes of a 615 bp ND2 gene fragment across both *Actophilornis* species. Of these, two haplotypes were exclusive to The Gambia and a single haplotype represented all Madagascar jacana samples (Figure 4.8). The remaining seven haplotypes were distributed among the African jacana sampling sites (Figure 4.8). Nucleotide diversity per site was low (π = 0.002 s.d. 0.0003) and haplotype diversity was highest in the Gambia (0.73 s.d. 0.16), followed by Botswana (0.57 s.d. 0.06), Zimbabwe (0.54 s.d. 0.03), South Africa (0.40 s.d. 0.06), Mozambique (0.32 s.d. 0.081), Uganda (single haplotype) and Madagascar (single haplotype).

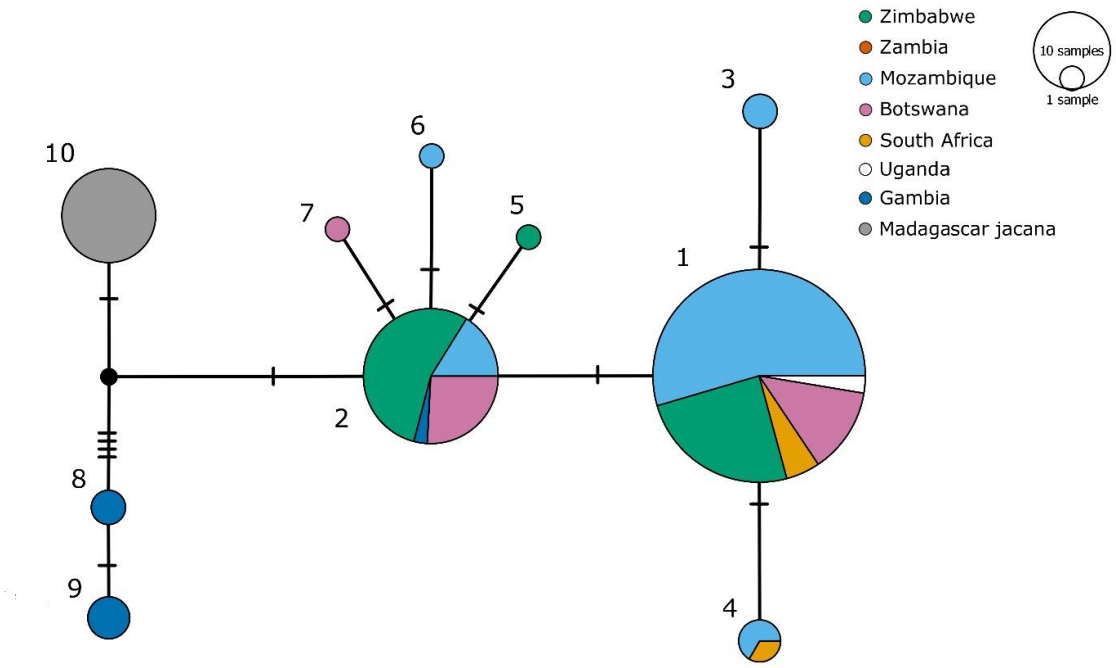

Figure 4.8. A haplotype network of Madagascar (*Actophilornis albinucha*, grey) and African (*Actophilornis africanus*, all other colours) jacanas, created using the Templeton, Crandall and Sing (TCS, 1992) method in PopArt (Clement *et al.* 2002). The network was created from a 615 bp fragment of the ND2 mitochondrial gene and the numbers represent each haplotype (n=10).

Inter- and intra-specific sequence divergence

Inter- and intra-specific divergence was higher with the autosomal consensus of RAD loci compared to the Z chromosome consensus of RAD loci (Table 4.4). The highest pairwise genetic distance with the autosomal scaffold was between Madagascar jacana and southern African jacana, whereas, with the Z chromosome scaffold, this pairwise comparison showed the lowest genetic distance (Table 4.4). In contrast, pairwise distance of mtDNA was highest between Madagascar jacana and Gambian African jacanas (0.008), followed by southern African jacanas and Gambian African jacana (0.007), and lowest between southern African jacana and Madagascar (0.006). Overall, we found discordant patterns of pairwise genetic distances among the mtDNA, autosome and Z chromosome sequences (Table S4.7).

Table 4.4. Pairwise mean genetic distance (Maximum Composite Likelihood method, Tamura *et al.* 2004) between the African (*Actophilornis africanus*) and Madagascar jacana (*Actophilornis albinucha*) consensus RAD loci within one autosomal and one Z-linked scaffold (grey shaded), calculated in MegaX (Kumar *et al.* 2018). African jacana is divided into two genetic clusters, The Gambia and southern Africa (see Figures 4.3-4.5).

	African (southern)	**African (The Gambia)**	**Madagascar**
African (southern)	0	0.169	0.178
African (The Gambia)	0.114	0	0.164
Madagascar	0.104	0.165	0

Discussion

Here we comprehensively assessed the genomic signatures of classical polyandry in *Actophilornis* jacanas using a combined marker approach. Supporting our hypotheses, we show that in both *Actophilornis* species, are characterised by high Z chromosome diversity and signals of population expansions. We found mixed support that polyandry promotes female biased dispersal and male site fidelity (Küpper *et al.* 2012, D'Urban Jackson *et al.* 2017) using comparative spatial genetic analyses of Z chromosome, autosomal and mtDNA markers. Finally, consistent with our hypothesis, we found that polyandry was not associated with 'Fast-Z' evolution (Mank *et al.* 2007), rather, intra- and inter-specific divergence was greater based on RAD sequences from an autosomal scaffold compared to those from a Z chromosome scaffold.

Effective population size

We found that genetic diversity was greater in the African, compared to the Madagascar jacana. Lower diversity of island species or populations compared to their mainland relatives is common across taxa (e.g. Frankham 1997, Johnson *et al.* 2009, Hughes 2010, Wang *et al.* 2014, but see: García-Verdugo *et al.* 2015), and could have been caused by having few founding individuals, a small effective population size, a high rate of inbreeding and/or stronger genetic drift (Frankham 1997, 1998). Supporting the role of a smaller population size causing lower genetic diversity, the Madagascar jacana has a smaller census population size compared to the African jacana (Madagascar: 1000-2000 individuals, Chapter 5, African: 1,000,000 individuals, Birdlife International, 2016). Furthermore, using ABC, the estimations of the Madagascar jacana current N_e were approximately half that of the African jacana. However, the confidence intervals (95% highest probability density) of N_e in were very wide for both species, covering 93-96% of the prior distributions. Wide confidence intervals are common in ABC analyses with fewer than 10,000 loci (e.g. Robinson *et al.* 2014, Shafer *et al.* 2015), therefore, these N_e estimations should be interpreted with caution.

Alternatively, lower genetic diversity of the Madagascar jacana could suggest that the frequency of polyandry is less in the Madagascar jacana compared to the African jacana. Female promiscuity is associated with high genetic diversity (reviewed by Slatyer *et al.* 2012, Taylor *et al.* 2014) and studies of passerines have found that extra pair paternity occurs less often in island populations compared to mainland conspecifics, due to reduced sexual selection pressure (Griffith 2000). However, there is no evidence of such a shift in breeding tactic in island populations of any jacana species, therefore, it is unlikely that a change breeding behaviour has caused lower diversity in the Madagascar compared to the

African jacana. Furthermore, supporting our hypothesis that classical polyandry will increase Z chromosome diversity above neutral expectations, we found both African and Madagascar jacana exhibit extremely high N_Z/N_A ratios (>0.96).

To our knowledge, the estimates of N_Z/N_A ratios we report here are the first from simultaneously classically polyandrous species. The overall N_Z/N_A ratios ranged between 0.96-0.97 in the African jacana and 1.26-1.52 in the Madagascar jacana, making them among the highest so far described in birds (reviewed by Irwin 2018). The N_Z/N_A ratios we found here are also greater than that of the red necked phalarope (N_Z/N_A=0.69, Corl and Ellegren 2012), which, despite being sex-role reversed (male only parental care), exhibits a low frequency of polyandry (Whitfield 1990). We found only one avian example of a N_Z/N_A ratio >1.0, which is from an island population of the zebra finch (*Taeniopygia guttata*) with very low overall diversity (Balakrishnan and Edwards 2009). In this case, it has been proposed that having a small population size contributed to a high N_Z/N_A ratio of the zebra finch, by increasing their vulnerability to stochastic variation in genetic diversity (Allendorf and Luikart 2007, Irwin 2018). Our findings also suggest stochastic variation may have contributed to raising the N_Z/N_A ratio, as the Madagascar jacana (island endemic, small population size) ratios were consistently greater than those of the African jacana. However, stochasticity is unlikely to have driven the pattern of high Z chromosome diversity across *Actophilornis* species, as they both high N_Z/N_A ratios, yet the African jacana a widespread, common species (BirdLife International 2016). The N_Z/N_A ratios calculated across all scaffolds in the Madagascar jacana were also above the theoretical maximum N_Z/N_A ratio of 1.125, which is expected under high skew in female breeding success without selection (Charlesworth 2001). Therefore, we propose that the high N_Z/N_A ratio in *Actophilornis* jacanas results from three dominate factors 1) intense selection acting on females (Kvarnemo and Simmons 2013), 2) high variance in female breeding success relative to male breeding success and, 3) male biased adult sex ratio (Tarboton 1992a, 1995).

An elevated N_Z/N_A ratio could, however, also be driven by our methodological approaches. Irwin (2018) found that genomic studies utilising restriction enzymes (e.g. Irwin *et al.* 2018) tended to produce higher N_Z/N_A ratios than investigations using genetic markers obtained from alternative methods (but see Lavretsky *et al.* 2015). The reason for this is unknown and requires further investigation (Irwin 2018). In addition, it is possible that scaffolds were erroneously categorised as on the Z chromosome, which were actually autosomal. Autosomal diversity is expected to be greater than Z chromosome diversity because two autosome copies are carried by each sex, therefore, including autosomal RAD loci in the Z chromosome dataset would produce erroneously inflated Z chromosome diversity. Of the 55 *J. jacana* scaffolds putatively identified as Z-linked, 20 also produced high BLAST match scores to the W chromosome. Multiple BLAST hits of gametologs is expected due to Z and W chromosome homologues, which commonly occur (Smeds *et al.* 2015). However, females were excluded for Z chromosome analyses, therefore, it is unlikely that these scaffolds reflect true homology. In addition, when calculating Z chromosome diversity, including RAD loci within the pseudo-autosomal region (PAR) could result in high Z chromosome diversity values, therefore, leading to in inaccurate N_Z/N_A ratios (Wilson Sayres 2018). To ensure only non-PAR Z chromosome loci are included for future investigations, we could assess heterozygosity across males and females, under the assumption that both males and females can be heterozygous in the PAR, but females will all be homozygous in the non-PAR Z chromosome region (Cristofari *et al.* 2016).

Demography

Highly elevated N_Z/N_A ratio can also be caused by a population expansions which disproportionately increase the genetic diversity on sex chromosomes compared to autosomes (Pool and Nielsen 2007). Assessing the effect of demography on N_Z/N_A is rarely conducted and disentangling them from the effects of breeding behaviour or evolutionary processes occurring on the sex chromosomes (e.g. 'Fast-Z' evolution) can be extremely difficult (Van Belleghem *et al.* 2018). Nevertheless, both jacana species produced signals of expansion from autosome and Z chromosome SNP loci with ABC analyses. The parameter distributions of both species and chromosome datasets are unsuitable to predict the exact the timing of expansions as the confidence intervals span the prior distribution. Although, models that tested for an expansion 1000-100,000 years ago were better supported than those with an expansion occurring within the past 1-1000 years. Geological records indicate large fluctuations in freshwater habitat availability in both species' range's during the past 100,000 years, for example, in Northern Africa regular monsoon rainfall during the Holocene (past 11,700 years) would have increased wetland land cover (Mitchell 2013). In contrast, in tropical Africa during the Pleistocene (2,588,000YA to 11,700YA) there were several periods of prolonged drought (Cohen *et al.* 2007). Similarly in Madagascar, during the Pleistocene glacial period, conditions were dry and wetlands expanded in the west only during the last 5000 years (Burney *et al.* 2004). Consistent with previous studies that have used ABC methods to model island colonisation and subsequent expansion (Lopes and Boessenkool 2010), the effective population size expansion of the Madagascar jacana could represent the colonisation of Madagascar from mainland Africa, as *Actophilornis* species have only recently diverged (Chapter 5). Although timing the divergence between these species was not the aim of this study, employing ABC methods on RAD loci sequences to determine the timing of divergence (e.g. Shafer *et al.* 2015) would be an interesting avenue of future research.

Supporting the validity of population expansions from ABC modelling, autosomal Tajima's D values of both species were significantly negative, which can occur due to a population expansion after a bottleneck or a recent selective sweep and/or purifying selection (Tajima 1989b). In contrast, Z chromosome RAD loci produced positive values which may have resulted from a recent contraction in population size (Tajima 1989b). However, the total number of sites in our Z chromosome dataset are just 1% of the number of autosomal sites, and Tajima's D variance was high in the African jacana Z chromosome scaffolds. While these results should be interpreted with caution, they support our hypothesis that polyandry promotes a genomic signal of a population expansions (Pool and Nielsen 2007).

Spatial genetic patterns

We found a lack of intraspecific genetic structure in Madagascar, and African jacanas (when excluding The Gambia individuals) across all three marker types (Z chromosome, autosomal and mtDNA), which suggests high dispersal of both sexes of *Actophilornis* jacanas. However, with autosomal loci in the African jacana, we detected a weak signal of isolation by distance when including males and females, but not with males only. Furthermore, when including The Gambia individuals, autosomal and Z chromosome loci both show significant isolation by distance, but the relationship between genetic and geographic distance is stronger with autosomal loci compared to Z chromosome loci. Together, the reduced isolation by distance in male genetic markers, with and without The Gambia individuals, could suggest weakly male biased dispersal in the African jacana. However, the difference between autosomal and Z chromosome loci isolation by distance trends is negligible (Z chromosome m = 0.39,

autosomes $m = 0.42$). Alternatively, sex-biased dispersal may be too weak to be detected by the methods we used. For example, detecting male biased dispersal may have been affected by errors in identifying Z-linked scaffolds (see above) or due to few long distance dispersers obscuring the genetic signal (Mills and Allendorf 1996). Detecting female biased dispersal in ZW systems is challenging due to the unreliability of mtDNA to detect isolation by distance (Teske *et al.* 2018), in addition to the difficulties in isolating W chromosome loci using low depth and low coverage sequencing methods (e.g. RADseq), due its small size relative to the genome and lack of genetic variation (Smeds *et al.* 2015). Taken together we are unable to strongly support a clear signal of sex biased dispersal of either *Actophilornis* species using nuclear loci.

We suggest the isolation by distance trends found in African jacanas when including The Gambia individuals, are the combined result of significant population differentiation between The Gambia and southern Africa, and a large gap in sampling distribution (5000 km), which together give the appearance of strong isolation by distance. Uneven sampling distribution often biases the interpretation of spatial genetic patterns (Meirmans 2012, Bradburd *et al.* 2018), which is why we repeated tests of isolation by distance and admixture within a subset of evenly distributed sampling sites (southern Africa). MtDNA corroborated findings from nuclear spatial patterns by confirming strong haplotype divergence between The Gambia and the southern Africa jacana sampling sites. However, without further sampling we are limited in our interpretation of this finding i.e. whether a barrier to dispersal exists or if the allelic frequencies change gradually. The stepping stone distribution of natural and manmade freshwater wetlands in mainland Africa (Hughes *et al.* 1992) and Madagascar (Bamford *et al.* 2017) and nomadic movements of jacanas (Tarboton 1995), is likely to promote high gene-flow within both jacana species. Our results partially complement recent gene-flow comparisons of new world jacana species (Genus: *Jacana*) which described significant isolation by distance in *J. jacana* but not in *Jacana spinosa* (Lipshutz 2018), and no isolation by distance with mtDNA in either species (Miller *et al.* 2014b). In addition, genetic homogeneity or clinal variation rather than structure population structure is common in birds with high dispersal capabilities (Medina *et al.* 2018), and continental shorebirds are no exception to this (Oyler-McCance *et al.* 2008, Küpper *et al.* 2012, Verkuil *et al.* 2012, Rönkä *et al.* 2012, Eberhart-Phillips *et al.* 2015).

However, two African jacana samples collected in Uganda, which could represent an intermediate population between The Gambia and the southern Africa, share a southern African jacana haplotype. This suggests that a barrier to gene-flow may be present, promoting divergence between West Africa and the central/southern African jacana. Commonly proposed genetic barriers between central and West Africa, even for vagile species (e.g. birds and bats) are, The Dahoney Gap, which is an area of dry savannah dividing the equatorial forests between Ghana and Nigeria (Salzmann and Hoelzmann 2005), the Niger River, and the Congo River (Voelker *et al.* 2013, Fuchs and Bowie 2015, Fuchs *et al.* 2018b). However, other widespread African endemic bird species show alternative phylogeographic divisions such as a Northern/Southern African split as in the Fiscal Shrike (*Lanius collaris*) (Fuchs *et al.* 2011), altitudinal differentiation (Bowie *et al.* 2004), differentiation by bioregion (Fuchs *et al.* 2018a) or continental genetic panmixia (Peel *et al.* 2013).

Haplotype sharing of geographically distant populations (Uganda and southern African) and a lack of mtDNA diversity in the Madagascar jacana supports our hypothesis that polyandry promotes the proliferation of few mitochondrial linages (Shaw *et al.* 2018), and high dispersal of females (D'Urban Jackson *et al.* 2017). The low overall haplotype diversity

found is consistent with other jacana species (Lin 2005, Miller *et al.* 2014b), and more generally, other shorebird species (e.g. red knots, *Calidris canutus:* Buehler and Baker 2003, redshank, *Tringa totanus*: Ottvall *et al.* 2005). Overall, the uneven sampling distribution of the African jacana, and a lack of clear sex specific genetic patterns in either *Actophilornis* jacana species, limit our ability to resolve the association between classical polyandry and sex-biased dispersal.

Speciation and population sequence divergence

Our focus on classically simultaneous polyandrous species in this study contrasts to a growing number of studies where authors have described elevated divergence on the Z chromosome compared to the autosomes in monogamous and polygynous ZW species (Lavretsky *et al.* 2015, Dhami *et al.* 2016, Oswald *et al.* 2016, Van Belleghem *et al.* 2018). We found greater intra- and inter-specific differentiation with the consensus RAD sequences from an autosome compared to the equivalent from the Z chromosome. This supports our hypothesis that polyandry is not associated with increased population or species divergence on the Z chromosome compared to the autosome. We suggest that increased diversity of the Z chromosome strengthens purifying selection and reduces the opportunity for genetic drift, therefore, reducing intra- and inter-specific divergence on this chromosome (Laporte and Charlesworth 2002, Vicoso and Charlesworth 2009, Wright and Mank 2013, Wright *et al.* 2015). However, further investigation is required to examine the effect of polyandry on intra- and inter-specific divergence, because our results are based on comparisons of the genetic distance of sequences from just two scaffolds (one autosomal and one Z chromosome) which may not represent the whole genome. Furthermore, we found discordance between the pairwise genetic distances of Z chromosome, autosome, and mtDNA sequences which could indicate low power of the sequences used (Braun and Kimball 2002). The ND2 sequence divergence of *Actophilornis* jacanas was also less than that between the two extant *Jacana* species (1.8%) which are known to hybridise (Miller *et al.* 2014a). Overall, our findings suggest a more recent divergence of the African and Madagascar jacanas compared to other endemic Madagascan bird species and their mainland African sister species (Bloomer and Crowe 1998, Groombridge *et al.* 2002, Woog *et al.* 2008, Melo *et al.* 2011, Arbabi *et al.* 2014, Fuchs *et al.* 2015).

To conclude, here we describe support for a genomic signature of classical polyandry in *Actophilornis* jacanas. We suggest the high N_Z/N_A ratios in both jacana species is driven by male biased adult sex ratio and high female, compared to male, reproductive skew. However, further research is required to disentangle the proportional contributions of biased adult sex ratio, reproductive skew, in addition to population expansions, in elevating the Z chromosome diversity. Furthermore, to contextualise our findings, we encourage the inclusion of classically polyandrous species in future comparative investigations into the genetic consequences of mating systems. For example, comparing the diversity and population divergence of the African jacana compared with monogamous lesser jacana, *Microparra capensis*.

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Chapter 4 Supplementary Material

(I) *Permit information for samples caught for this study*

Sampling and export permits.
Import to the UK AHVLA: TARP 2015/283 ITIMP 17.1606, TARP 2015/083, U1180785/ABP/OTHER

South Africa KZN Ezemvelo: HO/4046/13, OP 473/2016, Madagascar: 002N-EA01/MG17

The Gambia: No permits required, contact Mwado Jallow Department of Parks and Wildlife Management, Abuko Nature Reserve HQ, KMC, The Gambia

Ethical permissions for field work:
University of Bath Approved by Chair of the Animal Welfare and Ethical Review Body (AWERB) Malcolm Hooley: M.Holley@bath.ac.uk (Madagascar approval date: 23/11/2015, South Africa approval date: 28/10/2015)

(II) *Population differentiation (F_{ST})*

To determine the presence of population differentiation in Madagascar jacana and African jacana we calculated F_{ST} in Arlequin v3.5.2.2. (Excoffier and Lischer 2010) from called genotypes, because calculating F_{ST} using the multidimensional site frequency spectrum method implemented in ANGSD is not recommended for folded site frequency spectrums (ngsTools tutorial https://github.com/mfumagalli/ngsTools/blob/master/TUTORIAL.md). To allow for direct comparisons between the Z chromosome and autosome SNP loci we included only males.

Autosomal and Z chromosome loci showed significant population differentiation (F_{ST}) between Gambia and all other African sampling sites (Table S4.1i). Very low to no differences were present between the southern African sampling sites with autosomal loci, whereas, larger differences were found between southern sites with Z chromosome loci (Table S4.1). In Madagascar jacana, populations were very weakly differentiated by F_{ST} for both genomic regions (Table S4.1ii). Z chromosome F_{ST} values should be interpreted with caution due for both species due to a low number of loci used (African n=58, Madagascar n=33) after the automatic removed of loci with >0.05 missing data, which is implemented in Arlequin.

Table S4.1. Population differentiation (F_{ST}) between African (*Actophilornis africanus*) (i) and Madagascar (*Actophilornis albinucha*) (ii) jacana sampling sites for autosomal and Z-linked SNPs (grey shaded). See Table 4.1 for sampling location codes. * p=0.05, **bold** p<0.05, underlined p<0.01. Note that South Africa is represented by a single individual.

i)

African	ZIM	BOT	MOZ	SAF	ZAM	GAM
ZIM	0	**0.01**	0	**<0.01**	0	**0.05**
BOT	0	0	<0.01	**<0.01**	<0.01	**0.05**
MOZ	0	0	0	0	0	**0.05**
SAF	0.02	0.13	0.07	0	0	**0.07**
ZAM	0	0.01	<0.01	0.09	0	**0.03**
GAM	**0.07**	**0.05**	**0.09**	0.02	**0.02**	0

ii)

Madagascar	1	2	3	4	5
1	0	**<0.01**	0	0	**<0.01**
2	0	0	0	0	**<0.01**
3	0	0	0	0	0
4	0	0	0	0	0
5	0.01	0	0	0.01	0

Table S4.2. Prior range values and rules of estimated parameters for three demographic scenarios tested with ABCtoolbox to assess effective population size change of Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas. All priors are transformed into $\log_{10}$ values. N_e = effective population size. Ancestral population refers to either 1-1,000 years ago or 1,000-100,000 years ago, both time points were simulated for each scenario.

Scenario	**Current N_e**	**Ancestral N_e**	**Rules**	**Mutation rate**
Stable	1 to 5	1 to 5	-	-6 to -2
Bottleneck	1 to 4	1 to 8	Current N_e < Ancestral N_e	-6 to -2
Expansion	1 to 8	1 to 4	Current N_e > ancestral N_e	-6 to -2

Table S4.3. The number of loci included in population genetic analyses of Madagascar (*Actophilornis albinucha*) and African (AJ, *Actophilornis africanus*) jacanas. X indicates the population(s) included. A = autosomal scaffolds, Z = putative Z chromosome linked scaffolds. Only males were included in direct comparisons of autosomes and Z chromosomes. Shaded cells indicate loci included in ABCtoolbox analysis of 20 high depth individuals from each species.

Population			Sex	Genomic region	N° loci
Madagascar	southern Africa (AJ)	The Gambia (AJ)			
X			male	A	4,868
X			male	Z	300
X			both	A	4,212
	X	X	male	A	4,645
	X	X	male	Z	414
	X	X	both	A	3,735
X	X	X	male	A	4,015
X	X	X	male	Z	364
X	X	X	both	A	3,207
	X		male	A	4,585
	X		male	Z	400
	X		both	A	4,178
X	X		male	A	9,231
X	X		male	Z	775

Table S4.4. Genetic diversity per scaffold and across all scaffolds of Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas. Θ_w = Watterson's theta, π = nucleotide diversity, $\sum$ = sum of all sites. * = corrected values for male mutation bias (multiplied by 1.1). Diversity statistics were calculated in ANGSD (Korneliussen *et al.* 2014) using a folded site frequency spectrum.

Species (genetic cluster)	Chromosome	Calculated per scaffold				Calculated across scaffolds				
		mean π* per site	mean Θ_w* per site	mean π per site	mean Θ_w per site	$\sum \pi$	$\sum \Theta_w$	$\sum$ all sites	overall π per site	overall Θ_w per site
African jacana (Gambia)	Autosomes	0.005	0.004	0.004	0.004	336188	305292	80221879	0.004	0.004
	Z chromosome	NA	NA	0.007	0.006	3779	3405	856379	0.004	0.004
African jacana (southern)	Autosomes	0.007	0.007	0.007	0.006	294902	279704	60513813	0.005	0.005
	Z chromosome	NA	NA	0.011	0.009	3882	3653	823369	0.005	0.004
Madagascar jacana	Autosomes	0.003	0.002	0.002	0.002	89722	105236	60563686	0.001	0.002
	Z chromosome	NA	NA	0.006	0.004	1502	1459	666347	0.002	0.002

Table S4.5. Marginal densities and the significance of simulated demographic scenarios of the Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas using autosomal and Z chromosome loci. Simulations were conducted with fastsimcoal v2 (Excoffier and Foll 2011) within an approximate Bayesian computation (ABC) framework using ABCtoolbox (Wegmann *et al.* 2010).

Scenario	Time period	Species	Marginal Density		*p*-value	
			Autosomes	Z	Autosomes	Z
Bottleneck	1-1,000	African	0	3.15e-150	0	0
Stable	1-1,000	African	0.26	0.26	0.65	0.72
Expansion	1-1,000	African	0	5.65e-91	0	0
Bottleneck	1-1,000	Madagascar	0	5.04e-56	0	0
Stable	1-1,000	Madagascar	0.31	0.33	0.05	0.85
Expansion	1-1,000	Madagascar	0	8.39e-55	0	0
Bottleneck	1,000-1,00,000	African	0	1.60e-91	0	0
Stable	1,000-100,000	African	1.67e-3	9.85e-4	0	0
Expansion	1,000-100,000	African	1.11	1.19	0.99	1
Bottleneck	1,000-100,000	Madagascar	0	2.13e-54	0	0
Stable	1,000-100,000	Madagascar	0.01	0.01	0.05	0.04
Expansion	1,000-100,000	Madagascar	1.11	1.06	0.99	0.99

Table S4.6. Estimated current and ancestral effective population size (Ne) and the time of expansion for Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacanas using autosomal (A) and Z chromosome (Z) loci. High density probability (HDP) 95% intervals are provided for each estimate. Chr = chromosome. Demographic simulations were conducted with fastsimcoal v2 (Excoffier and Foll 2011) within an approximate Bayesian computation (ABC) framework using ABCtoolbox (Wegmann *et al.* 2010).

Species	Chr	Current Ne		Ancestral Ne		Time of expansion	
		mode	HDP 95%	mode	HDP 95%	mode	HDP 95%
African	A	1,289,021	124,609-48,396,059	2,327	15-8,406	33,835	5,478-96,020
African	Z	1,382,738	122,580-48,323,680	1,702	13-7,574	71,146	3,985-94,528
Madagascar	A	620,226	43,282-29,810,550	311	15-8,118	59,205	3,964-94,029
Madagascar	Z	565,900	42,578-27,421,424	2,494	15-8,119	67,164	4,981-95,023

Table S4.7. Highest (1) to lowest (3) pairwise genetic distance (Distance) of Madagascar jacana (*Actophilornis albinucha*) and African jacana (*Actophilornis africanus*; two populations The Gambia and southern Africa) using mtDNA (ND2), Z chromosome and autosome sequences. Shading reflects pairs of comparisons. Z chromosome and autosome distances were calculated using consensus RAD loci sequences from one scaffold for each chromosome type.

Distance	mtDNA	Z chromosome	Autosome
1	Gambia vs Madagascar	Gambia vs southern Africa	southern Africa vs Madagascar
2	Gambia vs southern Africa	Gambia vs Madagascar	Gambia vs southern Africa
3	Madagascar vs southern Africa	Madagascar vs southern Africa	Gambia vs Madagascar

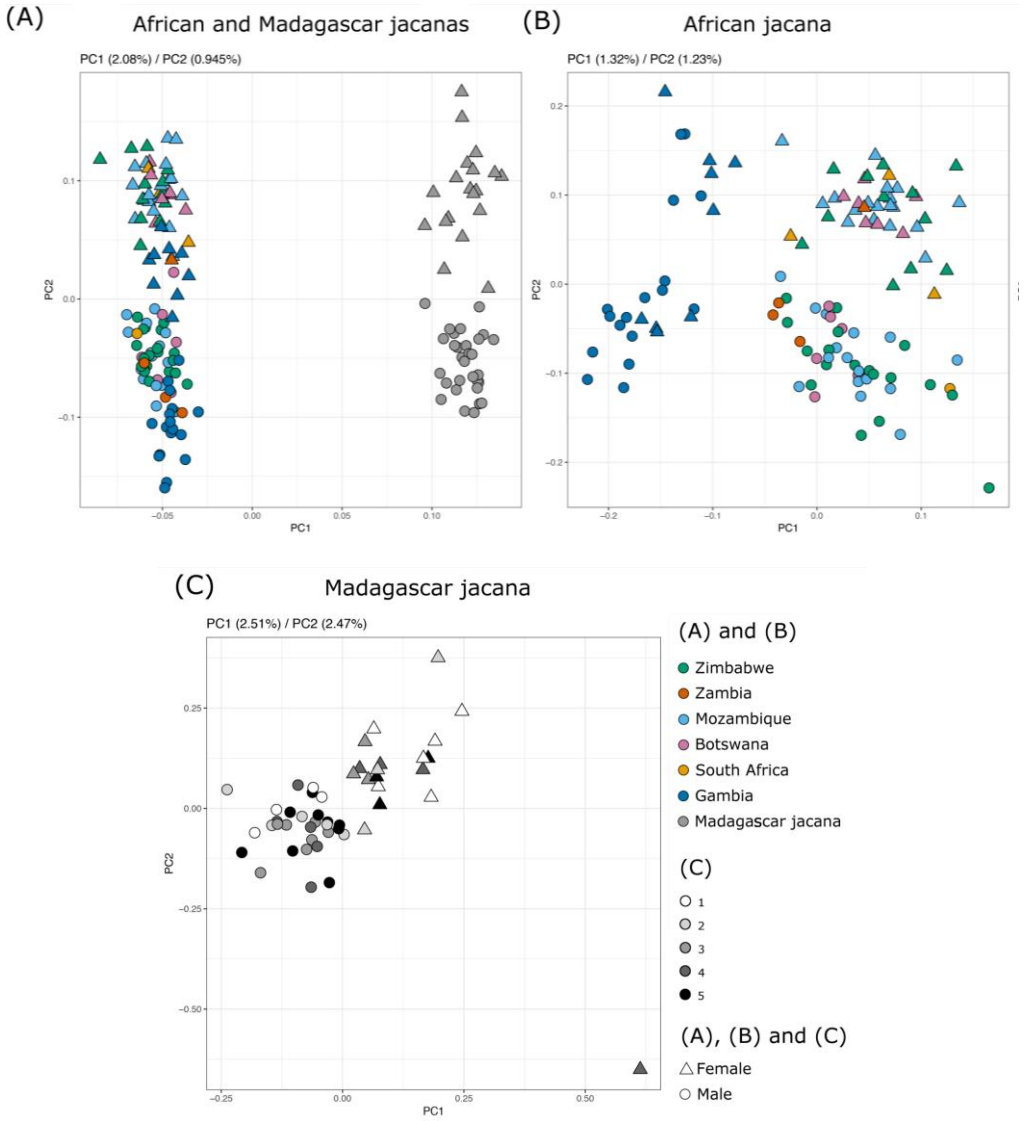

Figure S4.1. Principal component analysis of African jacana (*Actophilornis africanus*, A and B) and Madagascar jacana (*Actophilornis albinucha*, A and C) nuclear SNPs without filtering for loci out of Hardy Weinberg Equilibrium. Note the clustering of females (triangles) and males (circles).

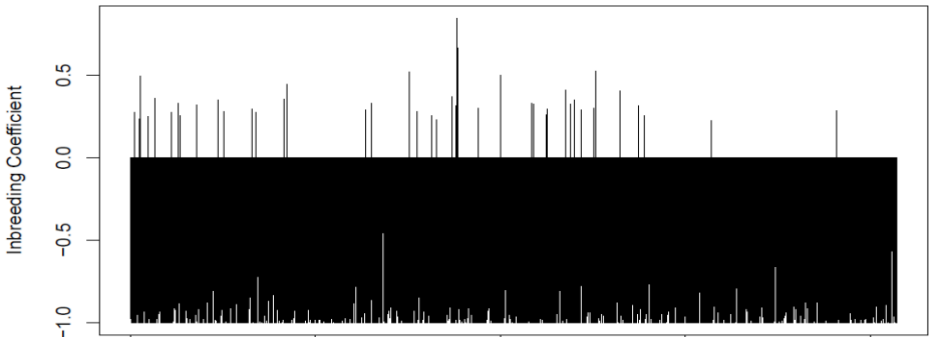

Figure S4.2. Inbreeding coefficient of each locus from restriction site associated DNA sequencing of African jacana (*Actophilornis africanus*) that was out of Hardy Weinberg Equilibrium.

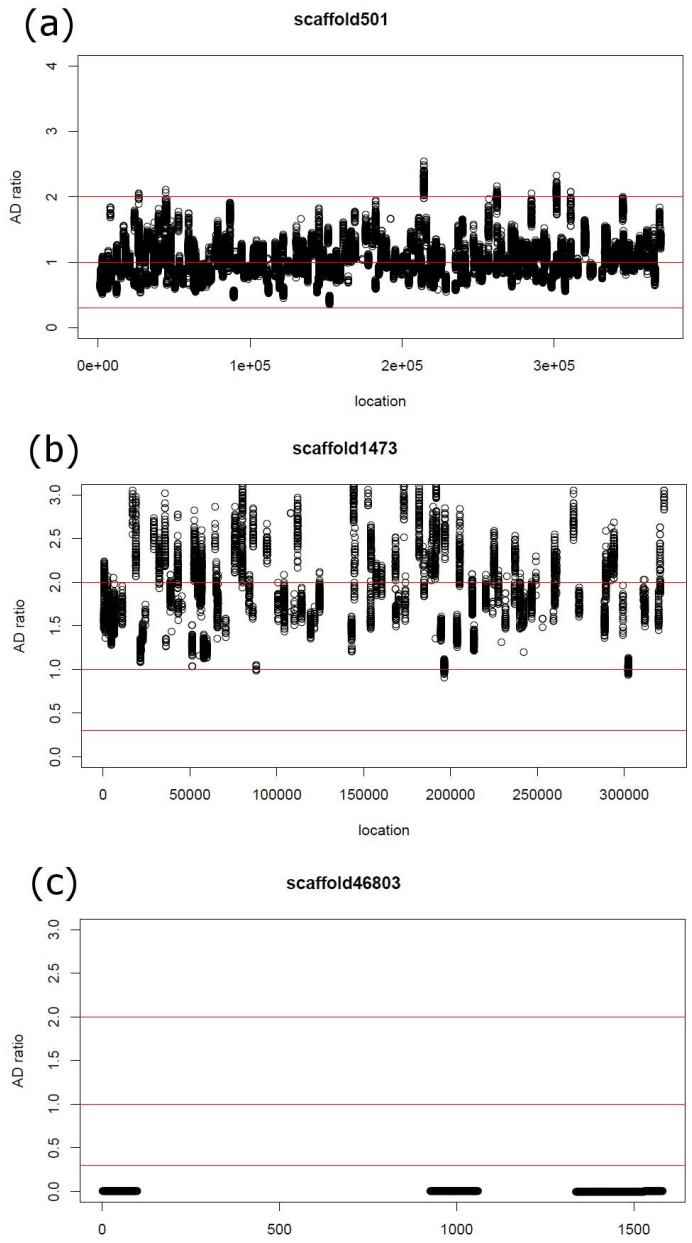

Figure S4.3. Locus depth ratio (AD ratio, male depth divided by female depth) of RAD loci positions on scaffolds representing (a) an autosomal chromosome, (b) a Z chromosome and (c) a W chromosome in southern African jacanas (*Actophilornis africanus*).

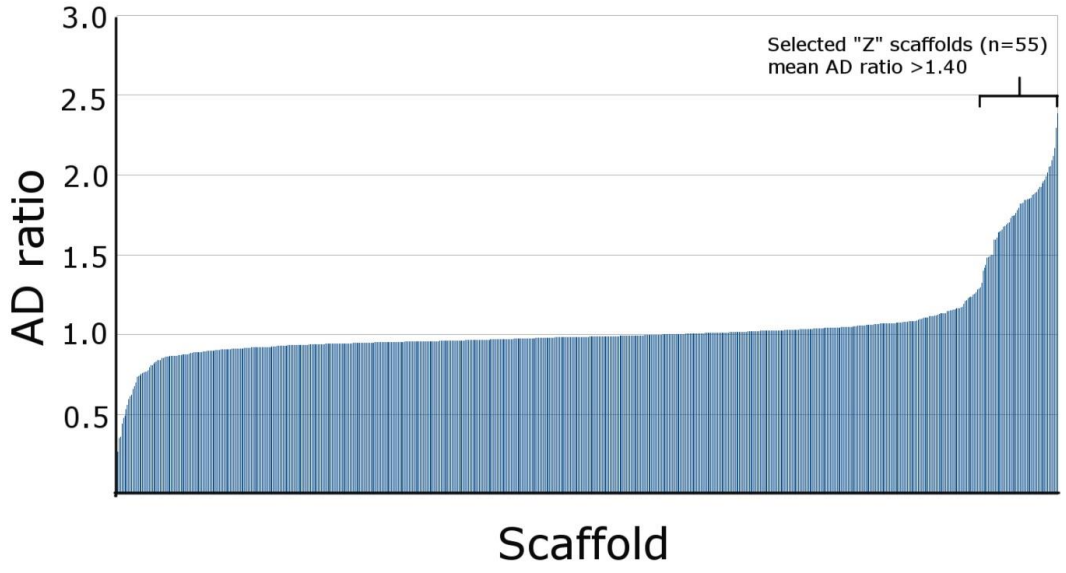

Figure S4.4. Average locus depth ratio (AD ratio, male depth divided by female depth) of Restriction site associated DNA sequences of African jacana (*Actophilornis albinucha*) from 687 scaffolds with high BLAST hit scores to Z chromosome gametologs of *Charadrius vociferous* (Zhou *et al.* 2014) and contained loci out of Hardy Weinberg equilibrium. Expected ratio of W chromosome: autosome: Z chromosome is 0: 1: 2. Indicated on the plot are the 55 scaffolds putatively identified as on the Z chromosome due to their high AD ratios.

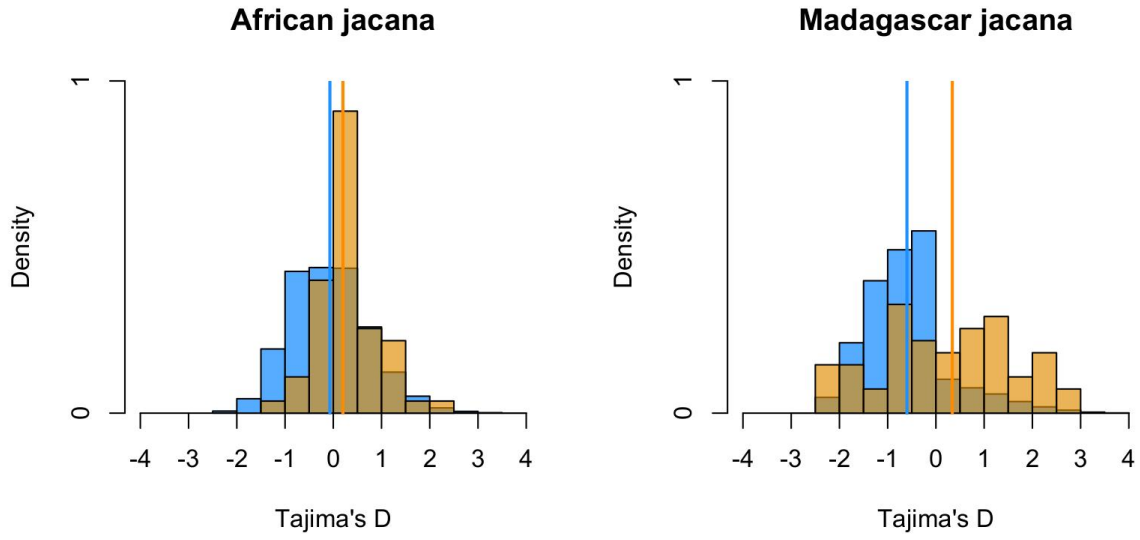

Figure S4.5. Density distribution of Tajima's D for Z chromosome (orange) and autosome (blue) scaffolds in African (*Actophilornis africanus*) and Madagascar (*Actophilornis albinucha*) jacanas. The average Tajima's D is indicated with a vertical line for each chromosome type.

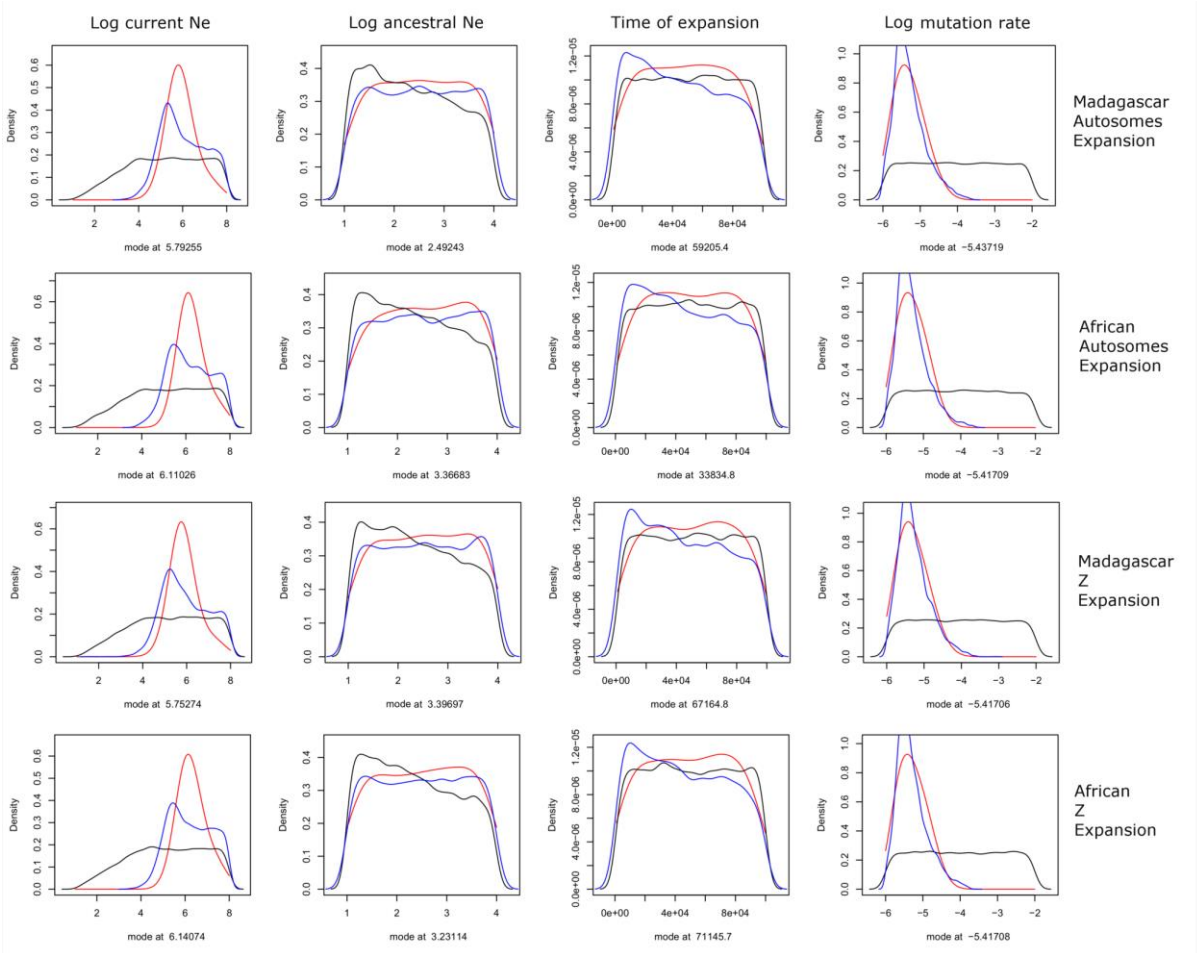

Figure S4.6. Posterior (red) and prior (black) distributions of demographic parameters of the best fitting scenario (expansion) estimated by approximate Bayesian computations for Madagascar (*Actophilornis albinucha*) and African (*Actophilornis africanus*) jacana in two genomic regions. Simulations were conducted with fastsimcoal v2 (Excoffier and Foll 2011) within ABCtoolbox (Wegmann *et al.* 2010). Blue lines represent marginal parameter distribution among the closest retained simulations (n=1,000) to the observed data. All parameter values in $\log_{10}$ scale.

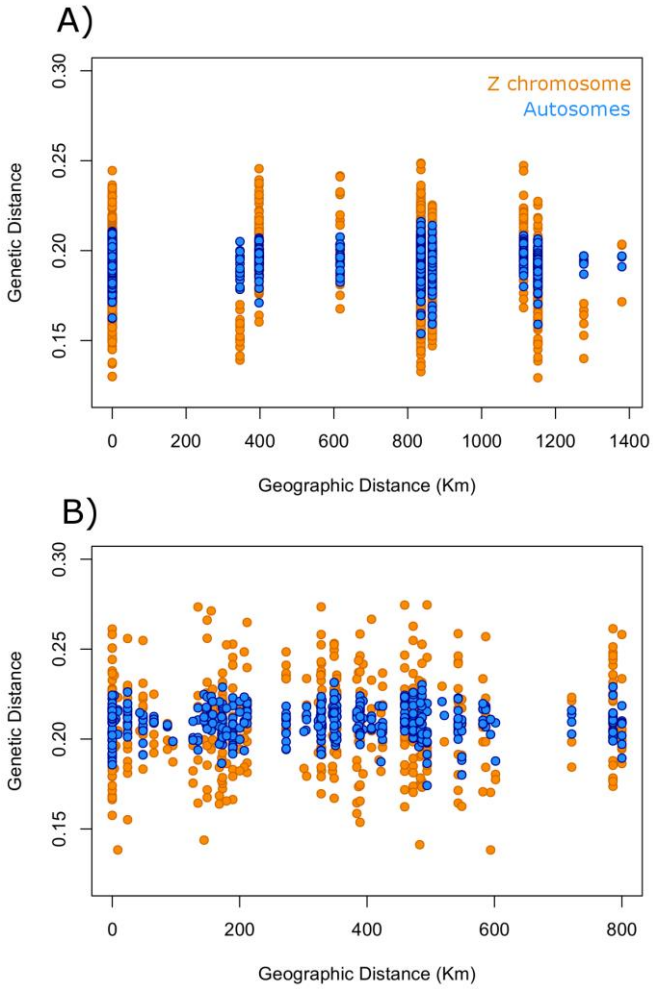

Figure S4.8. Individual pairwise isolation by distance of the African (*Actophilornis africanus,* A, southern populations only) and Madagascar jacanas (*Actophilornis albinucha*, B) for autosomal (blue) and Z chromosome (orange) loci. Only male individuals were included in these comparisons.

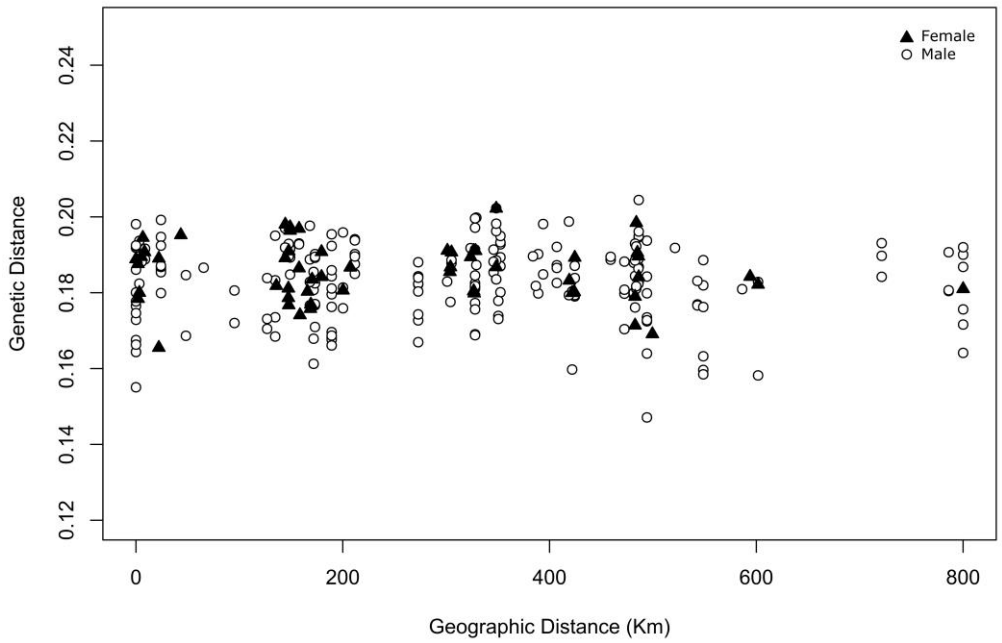

Figure S4.9. Pairwise isolation by distance of autosomal loci from adult males (circles) and adult females (triangles) of the Madagascar jacana (*Actophilornis albinucha*).

Commentary Post Chapter 4

In Chapter 4 I confirmed findings from Chapter 3, that classical polyandry is associated with high Z chromosome genetic diversity. Moreover, I have demonstrated in this chapter that simultaneous polyandry is associated with higher N_Z/N_A ratios compared with sequential polyandry (Chapter 3). High gene-flow was evident in both sexes and species with autosomal, Z chromosome and mtDNA markers. However, we only found weak support for the 'dispersal-to-mate' hypothesis (Chapter 2) based on low overall mtDNA diversity, which may suggest high female dispersal and skewed female reproductive success. To confirm the significance of this result, more female specific genetic markers are required.

Intra- and inter-specific divergence was lower on the Z chromosome than the autosomes, supporting the hypothesis that higher Z chromosome diversity reduces the opportunity for 'Fast-Z' evolution (Mank *et al.* 2007), we suggest this is achieved through more efficient purifying selection (Vicoso and Charlesworth 2009, Wright *et al.* 2015). This provides support for another mechanism by which sexual selection can reduce diversification, complementing findings from Chapter 2. Future studies should focus on disentangling the mechanisms behind increased Z chromosome diversity, as our findings could have resulted from population expansions (Pool and Nielsen 2007, Van Belleghem *et al.* 2018).

Commentary References

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Appendix B: Statement of Authorship

<table>
<tr><th colspan="10">This declaration concerns the article entitled:</th></tr>
<tr><td colspan="10">Distribution, ecology, and evolution of the Madagascar jacana</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td>X</td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td></td></tr>
<tr><td>Publication details (reference)</td><td colspan="9"></td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">J.D.J formulated the ideas for the project and designed the study with S.Z. and T.S. J.D.J designed and conducted fieldwork with S.Z. J.D.J. assisted with grant funding application for additional field work. J.D.J. conducted sexual size dimorphism analysis. J.D.J wrote the first draft of the manuscript. The manuscript has been improved further by co-authors. All figures were created by J.D.J.
J.D.J. contribution 80%</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
<tr><td>Signed</td><td colspan="6"></td><td>Date</td><td colspan="2"></td></tr>
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Commentary Pre Chapter 5

Madagascar jacana, *Actophilornis albinucha,* is an understudied wetland species endemic to Madagascar. In Chapter 4, I assumed that the Madagascar jacana is classically polyandrous based on their phenotypic similarity and geographic proximity to the African jacana (Jenni 1996, Safford 2013). However, in general we know very little basic information about this species. For example, the population size, population density and current distribution of the Madagascar jacana are based on poor quality data (BirdLife International, 2016). Wetland ecosystems, and the species that rely on them are highly threatened in Madagascar (Bamford *et al.* 2017, Máiz-Tomé *et al.* 2018), therefore, it is essential that efforts are made to understand the basic biology and distribution of wetland biodiversity, in order to make informed conservation decisions.

In this Chapter, I examine the distribution, ecology, and evolution of the Madagascar jacana. I apply phylogenetic analysis to create the first Jacanaidae phylogenetic tree with all eight extant species. In addition, as sexual size dimorphism is strongly associated with reversed sex roles, where females are markedly larger than males (Andersson 1994), I compare morphometric measurements of adult males and females to test the prediction that the Madagascar jacana is sex-role reversed (Jenni 1996, Safford 2013).

Commentary References

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Distribution, ecology, and evolution of the Madagascar jacana

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"I'm swanning round the world looking at the most fabulously interesting things. Such good fortune." **David Attenborough** (2013) *The Guardian*

Author contributions

J.D.J. Idea conception; funding; data collection; DNA extraction; conducted analysis; manuscript preparation
S.Z. funding; field work data collection; conducted analysis; manuscript improvement
S.M. field work data collection
A.J.B. conducted analysis (population estimate); manuscript preparation (population estimate); manuscript improvement
M.W.B. manuscript improvement
T.S. idea development; manuscript improvement

Distribution, ecology, and evolution of the Madagascar jacana

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Abstract

The Madagascar jacana (*Actophilornis albinucha*) is an understudied endemic shorebird found in the north and western Madagascan wetlands. Their habitat is highly threatened by human exploitation and wetland conversion to rice paddy fields, but the lack of basic knowledge of this species is hindering research and conservation efforts. The Madagascar jacana is predicted to exhibit the rare behaviour of sex-role reversal and classical polyandry, however, there is little data available to support this. Here, we conducted the most extensive study of this species to date focusing on their 1) distribution, population density, size, and trends, 2) degree of sexual size dimorphism (SSD), and 3) phylogenetic position. Surveys were conducted between January and October 2016, during which 58 lakes were visited. Madagascar jacana were found in 22 lakes and within these they were distributed at low densities, averaging 3.5 (Std. error 0.74) jacana per hectare of surveyed habitat. The number of jacana observed increased with size of the surveyed lake area. We confirmed that a substantial threat to this species is habitat destruction, however, existing monitoring data is not adequate to calculate population trends. We estimated the total population size at between ca. 1,000 and 2,000 individuals. Females were significantly larger than males, corroborating the large SSD described for the remaining six sex-role reversed jacana species (Family: Jacanidae). Using a 412 bp Cytochrome B fragment, we expanded the previous Jacanidae genetic phylogeny, and confirmed that the Madagascar jacana is a sister species to the African jacana (*Actophilornis africanus*). We advocate a thorough re-assessment of the threat status of the Madagascar jacana, and future work should include field studies on the breeding biology of this species.

Keywords: Madagascar jacana, Jacanidae, Actophilornis, Madagascar, wetland, shorebird, conservation, mtDNA, phylogeny.

Introduction

In the biodiversity hotspot of Madagascar (Myers *et al.* 2000), the total surface area of freshwater wetlands make up just 0.003% of land cover (Bamford *et al.* 2017) and are considered as highly threatened habitats due to land use change, specifically the conversion of wetlands to rice paddy fields (Benstead *et al.* 2003, Kull 2012, Bamford *et al.* 2017). The severe degradation of Madagascar wetlands has been predicted to have catastrophic impacts on the biodiversity within these ecosystems, and currently there are very few areas offering protection for wetland biodiversity (Bamford *et al.* 2017). As highlighted by the most thorough assessment of freshwater biodiversity in Madagascar to date, 43% of species are currently considered threatened (Máiz-Tomé *et al.* 2018).

The Madagascar jacana (*Actophilornis albinucha*) is one of 18 endemic wetland birds in Madagascar. Its population is estimated at ca. 1,000-10,000 individuals, however, the data quality of this prediction is poor (BirdLife International 2016). The current distribution of the Madagascar jacana stretches almost the entire length of the west Madagascar coastal wetland region (Safford 2013, Figure 5.1). However, the distribution records that the current range was based on are now out of date (some from over 100 years ago). Futhermore, they do not consider the huge transformation Madagascan land use has gone through in recent years, which includes a 60% reduction in wetlands (Kull 2012). Although Madagascar jacana was previously common in the west Madagascan wetlands (Safford 2013, BirdLife International 2016), recent anecdotal evidence of reduced sightings suggests this in unlikely to still be the case (BirdLife International 2016). As a result, in 2016 its threat status was elevated from "least concern" to "near threatened" due to declining population trend as a result of hunting, habitat loss and wetland degradation (BirdLife International 2016). This species currently receives no level of protection and, to our knowledge, like many wetland species which are understudied (Darwall *et al.* 2011) there has been no study conducted focussing directly on Madagascar jacana. Our lack of basic knowledge of this species is hindering further research and conservation efforts which require up-to-date distribution and population size estimates (IUCN 2001, Buckland *et al.* 2008).

Species of the family Jacanidae, jacanas, are highly adapted to freshwater aquatic environments (Emlen *et al.* 2004). The eight extant jacana species have colonised tropical and subtropical wetlands globally (Jenni 1996). Despite sharing ecological similarities, they have shown a mixed response to recent anthropogenic land use change (Okes *et al.* 2008, Pierluissi 2010). For example, the Africa jacana (*Actophilornis africanus*) is known to take advantage of man-made wetlands such as sewage works (B. Taylor pers. comm.), adapt to invasive species (Jenni 1996), and has shown signs of population expansion (Okes *et al.* 2008). Whereas, the lesser jacana (*Microparra capensis*) is more inconspicuous and is predicted to have suffered recent declines in population size (Okes *et al.* 2008).

Evaluating the basic ecology and conservation status of Madagascar jacana is difficult for at least four reasons. First, jacanas live and breed in areas with poor accessibility, especially in Madagascar, where wetlands are often inaccessible during the rainy season, which is when the species breeds (Safford 2013). Second, jacanas disperse frequently in response to changing environmental conditions (Jenni 1996, Tarboton 1995), therefore, assessing population size and distribution is difficult due to haphazard records (Runge

et al. 2015). Third, Madagascar jacanas lead an inconspicuous life-style, foraging and breeding in dense vegetation, which adds complications to ecological and behavioural data collection. Fourth, the Madagascar jacana often share their habitat with potentially dangerous species such as Nile crocodile (*Crocodilus niloticus*) and waterborne disease causing parasites (Genus: *schistosoma*, WHO 2012), therefore, trapping opportunities and behavioural observations are limited.

Simultaneous classical polyandry is defined by a reduction, or complete lack of, female parental care, combined with rapid multi-clutching (Emlen and Oring 1977, Graul 1977, Andersson *et al.* 1994). Classical polyandry is rare in nature (reviewed by Andersson 1995, 2005, Eens and Pinxten 2000, Owens 2002, Kvarnemo and Simmons 2013), occurring in just 1 % of bird species (Cockburn 2006, Lack 1968). Therefore, understanding the evolution of classical polyandry relies heavily on jacanas (Emlen *et al.* 2004), which, with the exception of the monogamous lesser jacana, provide many of the best studied examples of this unusual breeding behaviour (Jenni and Collier 1972, Vernon 1973, Tarboton and Fry 1986, Thong-aree *et al.* 1995, Jenni 1996, Butchart 2000, Mace 2000, Emlen *et al.* 2004). However, Madagascar jacana has so far been neglected, and classical polyandry has only been inferred from a few behavioural observations of the species (Jenni 1996, Safford 2013). Sexual size dimorphism (SSD) can be predictive of mating systems in shorebirds, with larger females relative to males often found in polyandrous species (Székely *et al.* 2000). Supporting this, female polyandrous jacanas are markedly larger than males (Jenni and Collier 1972, Johnsgard 1981, Tarboton 1995, Jenni 1996, Butchart 2000, Mace 2000, Emlen *et al.* 2004). Morphometric data for the Madagascar jacana is, however, based on just a few individuals that were not molecularly sexed (Safford 2013). Therefore, to confirm SSD, additional morphometric measurements, combined with a genetic sexing approach is required.

Consistent with many Old World tropical avifauna (Reddy 2014), we are not aware of any publically available Madagascar jacana phylogenetic data. As a result, there is currently no molecular phylogeny including all eight Jacanidae species (Whittingham *et al.* 2000). Understanding the evolutionary relationships between jacana species provide insights into the origin of Madagascar's endemic avian diversity (Reddy *et al.* 2012). Furthermore, phylogenetic information can improve conservation planning by identifying areas of high biodiversity value (Buerki *et al.* 2015, Chaudhary *et al.* 2018). There is a particularly urgency for well-informed conservation management in Madagascar, given its status as a biodiversity hotspot that is under severe anthropogenic threats (Brooks *et al.* 2006, Kremen *et al.* 2008, Jantz *et al.* 2015, Chaudhary *et al.* 2018).

The objective of this study was to conduct a thorough ecological assessment of the Madagascar jacana. Our aims were as follows: 1) assess the population trend of Madagascar jacana using wetland bird surveys conducted over the past 18 years, 2) conduct surveys to estimate the current distribution and population size, 3) determine the degree of SSD and compare morphometric measurements with the African jacana and 4) provide the first molecular phylogeny including all eight Jacanidae species.

Methods

Population trends

To provide baseline Madagascan jacana population trend estimates we extracted records of this species from wetland bird surveys conducted within 11 lakes between 2001-2016 (Table S5.1). Data from Madagascar wetland bird surveys were provided by Durrell Wildlife Conservation Trust (Richard Lewis pers. comms.), The Peregrine Fund (Lily-Arison Rene de Roland pers. comm., Razafimanjato *et al.* 2015) and the present study (see below). The number of Madagascar jacana were estimated visually from either multiple positions or a single location at each lake. Although it is inaccurate to combine data collected using different methods (multiple and single point count estimates) to estimate population trends (Buckland *et al.* 2008), we did this due to the paucity of long term data available for the Madagascar jacana. In doing so, count data from a minimum of 3 and a maximum of 10 repeated surveys per lake were collected (Figure S5.1A).

Madagascar jacana surveys

To collect data on the behaviour, current distribution, population size, and SSD of the Madagascar jacana, we conducted surveys between January and October 2016. Surveys were focused in lakes that had either 1) past Madagascar jacana sightings; 2) anecdotal evidence from local people of its presence and/or 3) suitable habitat e.g. freshwater lakes with floating vegetation (Safford 2013) (Figure 5.1 and Table 5.1). On arrival, each lake was visually surveyed once, starting at approximately 08.00 and lasting between one and three hours. We used two canoes to complete surveys with one person counting in each canoe using 10x8 binoculars. We minimized double counting by verbal communication between the two observers. Where possible, the entire perimeter of the lake was surveyed, however, in larger lakes e.g. Lake Bemamba, only locations with suitable habitat were visited. We calculated the total area surveyed at each lake by multiplying the length by the width using a GPS (Garmin GPS MAP62 ST). In larger lakes we calculated the size of each section of suitable habitat and summed them together to give one survey area estimate per lake. Annual rains lead to large fluctuations in the lake sizes and the area of suitable habitat for wetland birds in Madagascar (Young *et al.* 2005), therefore, collecting more exact measurements at these single time points was not carried out.

The number of adults (identified by full adult plumage; Safford 2013) and immatures (including intermediate plumage, Figure S5.2, Safford 2013) were recorded at each lake by visual confirmation. Habitat characteristics were recorded descriptively, including the level of human or livestock disturbance, presence of invasive water hyacinth (*Eichhornia crassipes*), and presence other wetland bird species. Where Madagascar jacana were found foraging, we observed their behaviour for 10-20 minutes before setting up mist nets in an arc shape above the water surface, around the foraging area. The Madagascar jacanas were enticed into the mist nets by paddling slowing towards them in the direction of the mist nets. We also searched for nests on the periphery of the lakes and when found, we measured the width and length of each egg with sliding callipers. Morphometric measurements were recorded for each bird caught following de Beer *et al.* (2001). We measured wing and tarsus length with a wing ruler, whereas,

shield width and length, head length and beak length were measured with sliding callipers. Body mass was measured with a 500 g Pesola ™ spring scale or a when this was not available, a digital pocket scale (0.01g accuracy). We took photographs of the head, neck, and body on top of a grey card (Figure S5.2) and recorded body condition (e.g. the presence of parasites, any injuries, feather damage and moult stage) for all Madagascar jacanas caught. For genetic sexing and mitochondrial (mt)DNA sequencing, we collected 25 µl of blood (stored in Queen's Lysis Buffer, Seutin *et al.* 1991) from each bird by puncturing the brachial vein with a 25 G needle (Owen 2011). Finally, we fitted a unique colour (n=3) and metal ring (n=1) combination to the tibiotarsus (two on each leg) for future population monitoring.

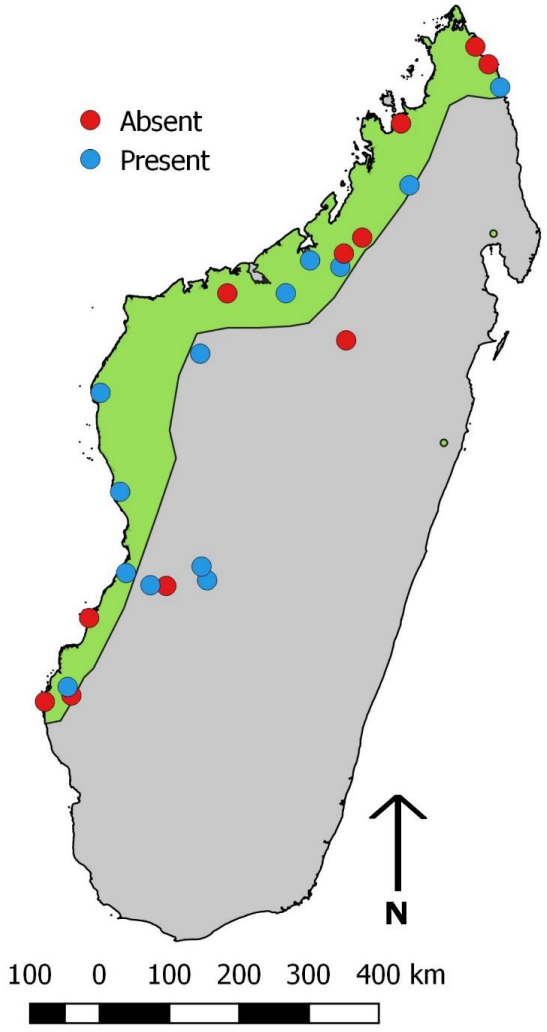

Figure 5.1. Map of areas surveyed in Madagascar between January and October 2016 for the Madagascar jacana (*Actophilornis albinucha*). Blue circles represent lakes where Madagascar jacana were found, and red circles represent lakes from where jacanas were absent, at the time of surveying. Lakes within close proximity of each other are combined as one area for visual simplicity. Green shading shows the species range according to BirdLife International (2016).

Table 5.1. The location (long=degrees longitude; lat=degrees latitude) of lakes occupied by Madagascar jacana (*Actophilornis albinucha*) during surveys conducted between January and October 2016. The surveyed area within each lake is presented in addition to the total number of jacana observed. Density was calculated by dividing the total number of individuals observed by the area surveyed.

Sites	Long	Lat	Area surveyed (ha)	Density (individuals / ha)	Total N° individuals (immature, adult)
Lake Anosy	49.90	-13.14	0.39	5.13	2(0,2)
Lake Ambinagny	49.96	-13.13	0.17	5.88	1(0,1)
Lake Matsaborilava	47.56	-15.76	1.00	3.00	3(0,3)
Lake Tsinjomitondraka South	47.12	-15.67	10.37	1.25	13(5,8)
Lake Madiromilomboka	46.77	-16.15	8.77	0.46	4(1,3)
Lake Ampisaraha	46.76	-16.14	1.44	1.39	2(0,2)
Lake Marogoaky	46.77	-16.14	6.79	1.18	8(3,5)
Lake Bejio Est	44.12	-17.57	2.27	3.09	7(0,7)
Lake Bejio Ouest	44.11	-17.57	1.03	2.92	3(0,3)
Lake Ampiliravao	44.05	-17.55	2.87	0.35	1(0,1)
Lake Mokotobe	44.06	-17.55	1.63	3.06	5(0,5)
Lake Nosin'omby	44.07	-17.55	1.00	8.00	8(0,8)
Lake Betakilotra	44.04	-17.54	34.32	0.29	10(1,9)
Lake Bemamba	44.36	-18.84	8.00	1.50	12(1,11)
Lake Belinta	44.43	-19.05	2.00	5.00	10(1,9)
Lake Besitera	44.35	-19.04	0.60	6.67	4(3,1)
Lake Ranovorindagory	45.54	-20.13	1.75	1.71	3(1,2)
Lake Ambariratibe	44.79	-20.42	0.72	5.56	4(2,2)
Lake Berano	44.79	-20.41	0.99	2.02	2(1,1)
Lake Belalitra	45.62	-20.34	1.00	8.00	8(1,7)
Lake Allée de Baobab	44.41	-20.26	0.50	4.00	2(1,1)
Lake Andramagnokely	43.59	-21.89	3.62	6.35	23(6,17)
Total			**91.22**	**1.48**	**135**

Population density and size

The relationship between the number of individuals and survey area size was assessed in R statistical software v3.5.1 (R Development Core Team 2018) with a linear model after log transforming area size to normalise the residuals.

Population density for each lake was calculated by dividing the area surveyed by the total number of birds that were visually observed during the survey. The Madagascar jacana population size was estimated by calculating the total surface area of wetlands in the range of the jacana using data from Bamford *et al.* (2017). We included wetlands up to 113 km outside of the current range based on our survey findings (Figure 5.1, Figure S5.3). Jacanas only occupy areas of wetlands with floating vegetation (Safford 2013), which commonly corresponds to the periphery of the lakes. Therefore, to account for

this we first calculated the total area of suitable habitat as a 10 m border inside each wetland or lake. Second, as Madagascar jacana do not occur on all wetlands within their range, we reduced this total area. The most recent unbiased survey of wetlands in the Madagascar jacana's range (Young *et al.* 2014) found Madagascar jacana on 5 out of 34 lakes surveyed (15 %). To provide a total population size estimation, we randomly selected 15 % of lakes within the Madagascar jacana range, and extrapolated the overall population density of lakes occupied by jacana (1.48, see Results), across the randomly selected area. Random selection of sites was necessary because predicting suitable wetland vegetation for Madagascar jacana from satellite images is too unreliable (A. Bamford pers. comm.). This random selection procedure was then repeated 1000 times to give a range of population estimates.

Sexing

To infer SSD, we molecularly sexed all of the Madagascar jacanas caught. We extracted DNA from blood samples using an ammonium acetate method (Bruford *et al.* 1998). The sexes were determined by amplifying the Chromo Helicase DNA-binding gene (CHD) using the 2550 (5'-GTTACTGATTCGTCTACGAGA-3') and 2718 (5'-ATTGAAATGATCCAGTGCTTG-3') primer pair (Fridolfsson and Ellegren 1999). Within a final reaction volume of 10 µl we included: 2 µl of 5X Green GoTaq® Flexi Buffer, 0.8 µl of 25 mM $MgCl_2$, 0.5 µl of each primer at 10 pmol/µl, 0.2 µl of 10 µM dNTPs, 0.25 units of GoTaq® G2 Flexi DNA Polymerase, 1µl of DNA (approximate concentration 5-20ng/ul) and 4.95 µl of double distilled H_2O. The PCR reaction used the following thermal profile: initial denaturation at 94°C for 2 minutes, 40 cycles of 94°C (45 seconds), 48°C (45 seconds) and 72°C (45 seconds), and a final extension step for 5 minutes at 72°C. For failed PCRs, a second primer pair for the CHD gene was used: CHD1F (5'_TATCGTCAGTTTCCTTTTCAGGT_3') and CHD1R (5'_CCTTTTATTGATCCATCAAGCCT_3') (Lee *et al.* 2010). All PCR products were visualised on a 2 % agarose gel electrophoresed for 1 hour at 100 V. One single band represented a male and two bands represented a female (Fridolfsson and Ellegren 1999, Lee *et al.* 2010).

Sexual size dimorphism (SSD) and mating system

We compared adult Madagascar jacana sex differences in tarsus and wing lengths in 13 females and 22 males. To prevent inconsistencies in mass SSD calculations due to using two different weighing methods (see above), we restricted the mass SSD measurement to birds weighed with the Pesola scale only, this included eight males and four females. We calculated SSD index of the Madagascar jacana by using log(male/female) (Székely *et al.* 2007). In addition, to compare the sizes between the Madagascar and the African jacana, we obtained tarsus length, mass, and wing length measurements (using consistent methods) from 35 female and 29 male African jacana caught in Mozambique, Botswana and Zimbabwe, via the Southern African Ringing Scheme database (G. Cumming pers. comm.).

First, we assessed each measurement (tarsus length, wing length and mass) of both sexes and species for normal distribution using Shapiro-Wilk test. Second, we performed parametric t-tests or non-parametric unpaired Wilcoxon rank sum tests (when the distribution was not normal) to assess the significance between pairwise comparisons.

All statistical analyses were performed with R statistical software v3.5.1 (R Development Core Team 2018) unless otherwise stated.

Evolutionary relationships

We amplified a 412 bp region of the Cytochrome B gene (CytB) in two Madagascar jacana individuals. The primer pair for this region was designed by aligning Jacanidae CytB sequences obtained from Genbank (accession numbers: EF373117.1 (*Actophilornis africanus*); EU166999.1 (*Jacana jacana*); DQ485894.1 (*Jacana spinosa*); EF373135.1 (*Hydrophasianus chirurgu*); EF373144.1 (*Microparra capensis*); EF373137.1 (*Irediparra gallinacea*); KF289833.1 (*Metopidius indicus*), Whittingham *et al.* 2000) and selecting primers to amplify the area of overlap in all species. The primers we designed were JacCytBF 5'_TCCTCCTTCTAACACTCATAGCA_3' and JacCytBR 5'_TGCTGTTAGGGCTAATACGC_3'. For a final reaction volume of 20 µl we included 4 µl 5X Green GoTaq® Reaction Buffer, 2µl, $MgCl_2$, 0.5 µl dNTPs (10 mM), 0.5 µl of each primer (10 pmol/µl), 0.10 units of GoTaq® G2 Flexi DNA Polymerase, 11.4 µl double distilled H_2O and 1µl of DNA. We used the following thermal profile: 94°C for 1 minute 30 seconds initial denaturation, 36 cycles of 94°C for 40 seconds, 64°C for 40 seconds, 72°C for 1 minute and a final extension for 5 minutes at 74°C. Negative controls were included for all PCR reactions and visualised with the same gel conditions as stated above. We conducted all PCR reactions in a VeritiTM 96-Well Thermal Cycler (Applied BiosystemsTM). The PCR products were sequenced by Eurofins Genomics Sequencing Services on an AB1 platform. We trimmed sequences in Sequencher ® 5.1 (GeneCodes Corporation), and aligned all Jacanidae CytB sequences with the ClustalW aligner method BioEdit 7.1.11 (Hall 1999). DNAsp V5 (Rozas *et al.* 2003, Librado and Rozas 2009) was used to produce diversity statistics and estimate sequence divergence.

We created a Bayesian inference phylogenetic tree in MrBayes v3.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003), using the GTR + gamma substitution model which was identified as most suitable by the Smart Model Selection (SMS) tool in PhyML (Lefort *et al.* 2017). For consistency with the previous phylogeny (Whittingham *et al.* 2000), we used greater painted snipe (*Rostratula benghalensis*) as an outgroup (Genbank accession AF146623.1; Whittingham *et al.* 2000). The parameters used were as follows: 5,000,000 MCMC generations, sampling every 500 repeats, sump and sumpt burnin = 1,250,000. The tree was visualised in FigTree v1.4.3. (http://tree.bio.ed.ac.uk/software/figtree/). Madagascar jacana sequences will be deposited in GenBank upon manuscript acceptance.

Results

Population trends

We compiled counts of Madagascar jacana from wetland bird survey conducted at 11 lakes between 2001 and 2016 (Figure S5.1). These data indicated large within and between year population size variation (Figure S5.1; Young *et al.* 2005, Young *et al.* 2014, Bamford *et al.* 2017, Durrell Wildlife Conservation Trust pers. Comm., The Peregrine Fund pers. comm.). Data from the two most surveyed sites, Lake Mandrozo and Lake Bemamba (Figure S5.1B) show a trend of population decline. Nevertheless, in recent years, the only large population of Madagascar jacana has been identified in the Lake Mandrozo New Protected Area (Figure S5.1). However, the available data were patchy, with large temporal gaps and some estimates had no month of collection associated with

them. Furthermore, the methods used for estimations varied between surveys. For these reasons we are unable to provide reliable estimates of population trends with these data and chose not to present them in the main text (see instead Figure S5.1A and B).

Madagascar jacana surveys

We surveyed 58 lakes in 2016 for Madagascar jacana, representing the full breadth of their known range, in addition to adjacent areas of suitable habitat (Figure 5.1). A total of 135 jacana (adults and immatures) were found in 22 lakes, representing 91.22 ha and 38 % of lakes surveyed (Table 5.1, Table S5.2). Four of the 22 lakes with Madagascar jacana were outside of the range (BirdLife International 2016), with one lake situated 113 km east of the range extent (Figure 5.1). We provide mean and standard error values unless otherwise stated. In the lakes with jacana, the number of individuals per lake ranged from one to 23, with an average of 6.14 (1.3). We found that the number of individuals counted significantly increased with the size of the area surveyed (Figure 5.2; adjusted R^2 = 0.26; p = 0.009, df = 20). The density of Madagascar jacana in occupied lakes was on average 3.5 (0.74) per hectare (ha) of area surveyed and the density combining all occupied lakes was 1.48 per ha (Table 5.1). The total surface area of wetlands in the Madagascar jacana range, including those 113 km to the east, is 1154 km^2 with the periphery of these wetland summing to 43 km^2. Extrapolating our overall density figure (1.48) over this area would give a population estimate of ca. 6,300, however, this is likely to be an overestimate due to biases in our survey. Following Young *et al.*'s (2014) survey, in which jacana were found at only 15% of sites, and using our overall density of 1.48 birds per ha, we predict a population estimate that ranges from 975 to 2064 individuals, with a median estimate of 1423.

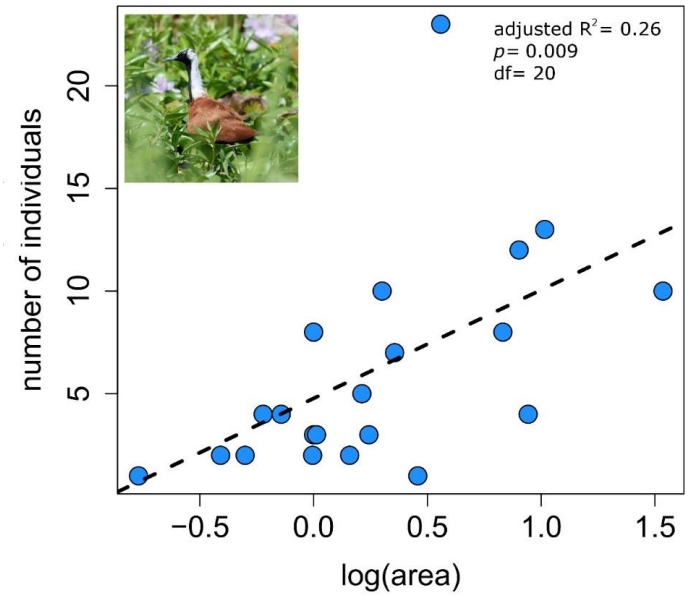

Figure 5.2. Linear relationship between the size of the lake area surveyed (n=22, log transformed, hectares) and the total number of Madagascar jacana (*Actophilornis albinucha*) individuals present, with regression line.

Threats and anecdotal reports of population trends

Human disturbance (e.g. washing, fishing, agriculture) was found at all sites with Madagascar jacana. The jacana were tolerant of people unless they were directly disturbed. We did not witness hunting of Madagascar jacana during our surveys, however, local people confirmed that waterbird hunting was common. Anecdotally, local people and bird tour guides described a steep decline in the number of Madagascar jacanas they had observed in recent years. During surveys we witnessed extensive and rapid destruction of natural wetlands for agricultural land. For example, the Lake Sahaka region (including Lake Anosy and Lake Ambinagny) in the North of Madagascar, was previously described as a permanent site for Madagascar jacana (Safford 2013), however, in our surveys we found only three individuals in this area.

Local people and non-Government Organisation (NGO) staff reported that many lakes only provide temporarily suitable habitat for Madagascar jacana due to seasonal weather fluctuations and agricultural practices (e.g. changes in the rice growing season, FAO 2016). For example, in Lake Bemamba, local people reported that following the rice growing season (January to April), the grasses and reeds provide dense cover for nest sites and chick rearing, during this time immature Madagascar jacana can be seen. After the rice harvest, we witnessed piles of rice reeds debris that provided foraging areas for adults. However, as these findings are based on anecdotal reports, we are unable to assess their accuracy until more thorough investigations into the specific threats to Madagascar jacana are conducted.

Sexual size dimorphism and egg size

Of the 135 Madagascar jacanas found, 55 were captured, which included 35 adults (22 male, 13 female) and 20 immatures (11 male, 9 female). Morphometric analysis revealed significant SSD, with mean female mass 60% heavier than male mass and mean wing length 15% longer (Table 5.2 and Figure 5.3). All morphometric t-test comparisons showed significant size differences between the sexes (Table 5.2 and Figure 5.3). The sexual dimorphism index ranged from -0.036 (tarsus) to -0.204 (body mass) (Table 5.2). Both sexes of Madagascar jacanas were significantly larger than the African jacana for mass, and wing length (mass: t = 3.08, df = 37, p-value <0.01, wing length: t = 8.45, df = 35.83, p-value <0.01), but not for tarsus length (t = 0.73, df = 46, p=0.464) (Figure 5.3). We found one nest containing three eggs during our surveys. The mean length of eggs was 33.4mm (std. dev. 0.3) and the width was 25.23mm (std. dev. 0.2). Molecular sexing of the brooding adult indicated it was a male. In the only other instance of an adult found with chicks, the adult was sexed as a female.

Table 5.2. Morphometric measurements (tarsus, wing and mass) of adult Madagascar jacana (*Actophilornis albinucha*) demonstrating sexual size dimorphism. Normally distributed data were compared with two sample t-test, whereas, Wilcoxon rank sum test were performed for non-normally distributed data. Sexual size dimorphism index was calculated as log(male/female) following Székely *et al.* (2007). SD= standard deviation; n= number of individuals included in the comparison; df= degrees of freedom.

	Tarsus		**Wing**		**Mass**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Min	62.7	64.3	139	180	145	240
Max	71.0	80.1	167	190	185	285
Mean	66.8	72.5	161.0	185.7	166.6	266.2
SD	2.64	5.54	6.86	3.63	14.70	18.87
N	22	13	22	13	8	4
"*t*" or "*W*" test statistic	3.46		264***		10.13	
p value	0.003		<0.001		<0.001	
df	15.29		NA		10	
log(male/female)	**-0.036**		**-0.062**		**-0.204**	

* Wilcoxon rank sum test

Evolutionary relationships

Phylogenetic analysis using CytB confirmed the Madagascar jacana is a sister species of the African jacana and together they represent a monophyletic clade (Figure 5.4). There were no sequence differences between the two Madagascar jacana individuals. Of the 421 bp region sequenced, four polymorphic sites were found between the Madagascar jacana and the African jacana.

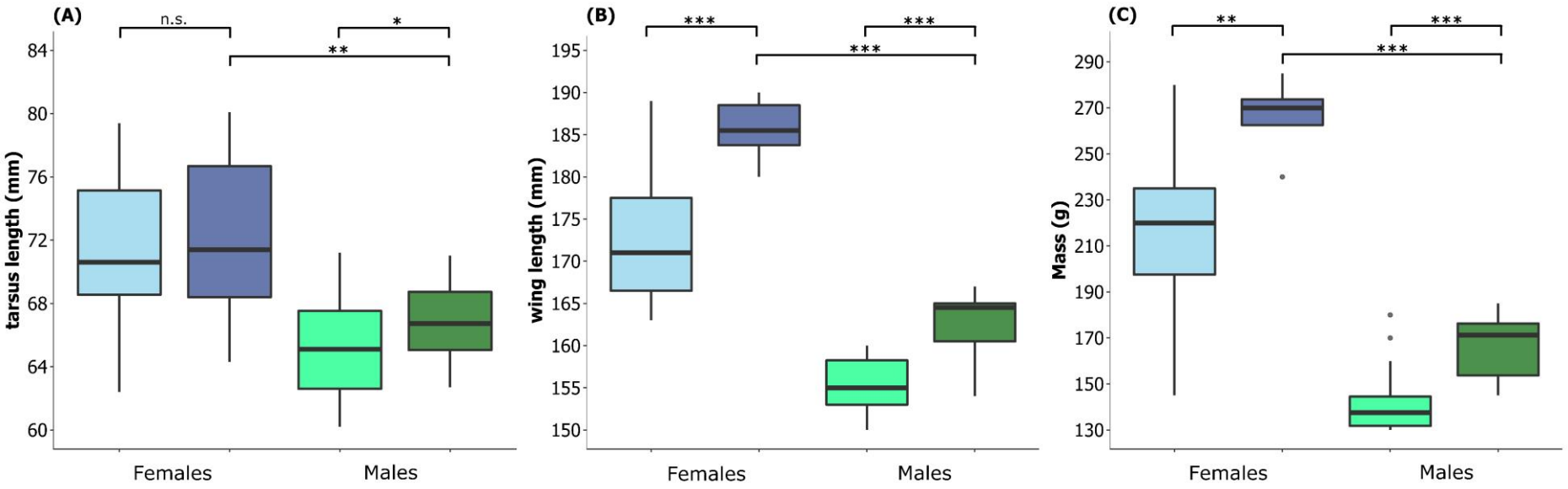

Figure 5.3. Adult morphometric measurements, (A) tarsus, (B) wing length, and (C) mass, of the African jacana (*Actophilornis africanus*; light blue = female; light green = male) and the Madagascar jacana (*Actophilornis albinucha*; dark blue = female; dark green = male). Tarsus and wing length comparisons include 35 Madagascar jacana (13 females and 22 males) and 64 African jacana (35 females and 29 males), samples size for mass of Madagascar jacana include 12 individuals (8 males, 4 females) and 64 African jacana (35 females and 29 males). Significance is indicated by: n.s. = not significant; * p <0.05, ** p <0.01, *** p <0.001

Figure 5.4. Phylogenetic relationships of all eight extant jacana species (Family: Jacanidae) created with a Bayesian Inference method using 421 bp of the Cytochrome B mitochondrial DNA gene. Posterior probabilities are shown for each node. One sequence of each species was included to create the tree. Outgroup = Greater painted snipe, *Rostratula benghalensis*.

Discussion

This study provides the first detailed account of the Madagascar jacana, an understudied endemic shorebird restricted to freshwater wetlands of Madagascar.

Distribution, population density, size and trends

Our findings largely support the estimated range of the Madagascar jacana, however, we suggest the current range should be extended eastwards to include additional suitable habitat. Shorebird range changes can be attributed to multiple interacting factors including food availability (e.g. Verkuil *et al.* 2012) and climate change (e.g. Bart *et al.* 2007). Alternatively, the African jacana has extended its range in recent years by 28% by taking advantage of novel man made wetlands (Okes *et al.* 2008). However, as there is little known about this species, it is likely that the range predicted by BirdLife International (2016) was simply too restrictive, rather than a range expansion. Due to the patchy distribution of Madagascar's wetlands and the ongoing transformation of the landscape (Benstead *et al.* 2003, Kull *et al.* 2012, Bamford *et al.* 2017), we argue that the Madagascar jacana range is not continuous and that the area of occupancy is likely to be

far smaller than the current range suggests. Our estimate, although very rough, was of just 43 km^2. For wetland specialist species, this discontinuous habitat distribution should be considered for conservation management by focusing on creating connected networks of wetlands (Amezaga *et al.* 2002, Haig *et al.* 1998).

Our findings suggest that this species is becoming increasingly vulnerable to extinction. However, estimating a reliable population size and trends is challenging for frequently dispersing species (Runge *et al.* 2015), especially for understudied species like the Madagascar jacana. Our estimate of ca. 1,000 to 2,000 individuals does not strictly represent a decline from the existing estimate of ca. 1,000 to 10,000 (BirdLife International 2016), rather, it focuses the estimate on the lower end of that range. Furthermore, with no reliable data on population trends it is difficult to reassess the jacana's status for the IUCN Red List criteria (IUCN 2001). Nevertheless, based on population size alone there are implications for the Red List status of the jacana, as there is an argument for it being classified as Vulnerable or even Endangered. We suggest more accurate population estimations are a high priority for future studies of the Madagascar jacana and these should include wetlands to the east of the current range boundaries. In addition, we strongly support the use of repeated surveys of wetland birds in Madagascar in order to calculate reliable population trends. The methods used in future repeated surveys should be consistent between researchers and organisations to allow results to be adequately compared (Buckland 2008). However, long-term monitoring of this sort is extremely difficult to fund and organise in developing countries such as Madagascar, meaning that population trends are uncertain even for well-known species (Murphy *et al.* 2017).

Repeated surveys in the Lake Mandrozo area (Latitude -17.56, Longitude 44.08) conducted by The Peregrine Fund suggest this site represents an important permanent habitat for the Madagascar jacana, with between 30-50 birds recorded each visit taking place between 2009-2015 (Figure S5.1, Razafimanjato and Randrianjafiniasa 2015). Additionally, a recent publication by Bamford *et al.* (2017) found 80 jacana in the Ambonara wetlands (Latitude -17.03, Longitude 45.52) in 2016 and 64 in 2017, along with a high diversity of wetland avifauna, suggesting this wetland requires long-term monitoring and urgent protection against land conversion.

There are at least three reasons which could explain the small number of jacana found during the 2016 surveys. Firstly, extreme departures from normal weather conditions caused by one of the strongest recorded El Niño events on record (Siderius *et al.* 2018) may have made previous suitable areas unsuitable during this year. Jacanas are known to disperse in response to wetland availability (Tarboton 1995, Safford 2013) and rainfall is highly variable in Madagascar (Tadross 2008, Macron *et al.* 2016). Therefore, the sites we visited in 2016 may not have provided optimal habitat at that time. Other jacana species occur naturally at low densities (Jenni 1996, Dostine and Morton 2000, Changder *et al.* 2015) which can make population surveys difficult, especially in areas of low accessibility.

Secondly, absences and low densities in areas with suitable habitat may be a result of interspecific competition, or predator avoidance as Madagascar jacanas were rarely observed alongside species with similar ecological niche (e.g. common moorhen (*Gallinula chloropus*) and Allen's gallinule (*Porphyrio alleni*)). In addition, we also

witnessed aggressive displays from Madagascar jacanas towards moorhens and gallinules. This supports the observation of aggressive interactions recorded between northern jacanas (*Jacana spinosa*) and purple gallinules (*Porphyrula martini*), which are known to predate northern jacana eggs (Stephens 1984).

Thirdly, the low density could be a result of a genuine population decline, as predicted from the most recent IUCN red list assessment (BirdLife International 2016). Unfortunately, the survey data we compiled was not suitable to draw reliable estimates of population trends from, which are urgently needed in order to re-assess the Madagascar jacana's threat status. During our surveys, anecdotal observations by local people and bird guides suggested a downwards population trend of the Madagascar jacana, similar to that of other Madagascan wetland bird species e.g. white-backed duck (*Thalassornis leuconotus insularis*) and the Madagascar grebe (*Tachybaptus pelzelnii*), of which many are classified as threatened (Bamford *et al.* 2017, IUCN 2017). Several factors are likely to be responsible for these population declines, most notably extensive wetland habitat destruction due to the conversion to agricultural land use, particularly rice (estimated to have caused the loss of between 60% and 82% of marsh habitat; Kull 2012, Bamford *et al.* 2017). The low number of individuals found in large lakes used for rice growing (e.g. Lake Bemamba and Lake Sahaka) suggests that rice paddy fields provide a suboptimal habitat for this species, but one which they can utilise for some parts of the year. The seasonal use of rice paddy fields by breeding and foraging waterbirds (including other jacana species) is common around the world (reviewed by Pierluissi *et al.* 2010, Marco-Méndez *et al.* 2015). However, the relative biodiversity value of these areas compared to natural wetlands is largely unknown (Fasola and Ruiz 1996, Sundar and Subramanya 2010, Antunes Dias *et al.* 2014), and is urgently required in Madagascar. One side effect of agricultural expansion is intensive pesticide use (Parsons *et al.* 2010). Jacanas are insectivores (Jenni 1996), therefore, pesticide use could affect their survival by reducing their food availability and/or have direct lethal effects (reviewed by Parsons *et al.* 2010). A population decline in Madagascar jacana could also be caused, or exacerbated by, reduced habitat availability a result of global climate change causing extreme fluctuations in rainfall (Tadross 2008). Unlike other Madagascan habitats (e.g. Raxworthy *et al.* 2008), there have been no projections of how climate change will effect freshwater biodiversity in Madagascar. This is despite a growing number of potential threats (Woodward *et al.* 2010) and a high level of vulnerability of this ecosystem (Bamford *et al.* 2017, Máiz-Tomé *et al.* 2018). Finally, we did not observe any direct persecution of Madagascar jacanas during our surveys, however, previous reports suggest this is an additional threat to their persistence (BirdLife International 2016).

Sexual size dimorphism and mating system

By combining genetic sexing with morphometric data we show that females are significantly larger than males, confirming previous SSD findings based on a small sample size (Jenni 1996, Safford 2013). Our finding suggests that like the six other jacana species with SSD, the Madagascar jacana is classically polyandrous and exhibits sex-role reversal (Jenni 1996; Jenni and Collier 1972, Vernon 1973, Thong-aree *et al.* 1995, Butchart 2000, Mace 2000, Emlen *et al.* 2004). However, dedicated breeding behaviour investigations are required to evaluate the generality of these observations, as sexual

size dimorphism is not always predictive of mating system (Székely *et al.* 2004). Furthermore, we observed female uniparental care of three immature birds, which may indicate that this species has a flexible mating system. We also found that the Madagascar jacana is larger than its mainland sister species, the African jacana, supporting the "Island Rule" (Whittaker and Fernandez-Palacios 2007). This theory predicts that island species evolve a larger body size than their mainland conspecifics (Whittaker and Fernandez-Palacios 2007). However, empirical support for this theory is mixed and the relationship between body size and other ecological factors is complex (Clegg and Owens 2002, Olson *et al.* 2009, Lokatis and Jeschke 2018).

Evolutionary relationships

As expected based on phenotypic similarity and geographic proximity, the molecular phylogeny of extant all Jacanidae species indicated the Madagascar jacana is a sister species of the African jacana, extending the previous phylogeny (Whittingham *et al.* 2000). The observed sequence divergence of 1% corresponds to a time of divergence of 500 KYA based on the "2% rule" for mtDNA (but see: Lovette 2004). This indicates a younger divergence of *Actophilornis* species in comparison with the divergence found between other Madagascan endemic avian species and their mainland African sister taxa, which range from 0.8MYA to 5MYA (Bloomer and Crowe 1998, Groombridge *et al.* 2002, Woog *et al.* 2008, Melo *et al.* 2011, Arbabi *et al.* 2014, Fuchs *et al.* 2015). However, our estimate of divergence is based on a short (412 bp) section of CytB and a single representative sample for each species which can result in inaccurate divergence estimates (Braun and Kimball 2002).

Conclusions

We present here a baseline study of the Madagascar jacana, which aims to improve our understanding of this endemic wetland bird. Many fundamental ecological and behavioural traits of the Madagascar jacana remain unknown, therefore, we encourage further investigation of this species, to build upon our study. In addition, ongoing threats to Madagascar wetlands and habitat destruction together suggest this species requires a targeted reassessment of its threat status and direct population monitoring.

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Chapter 5 Supplementary Material

Table S5.1. Location of 11 lakes where repeated wetland bird surveys took place in Madagascar, conducted by The Peregrine Fund and Durrell Wildlife Conservation Trust.

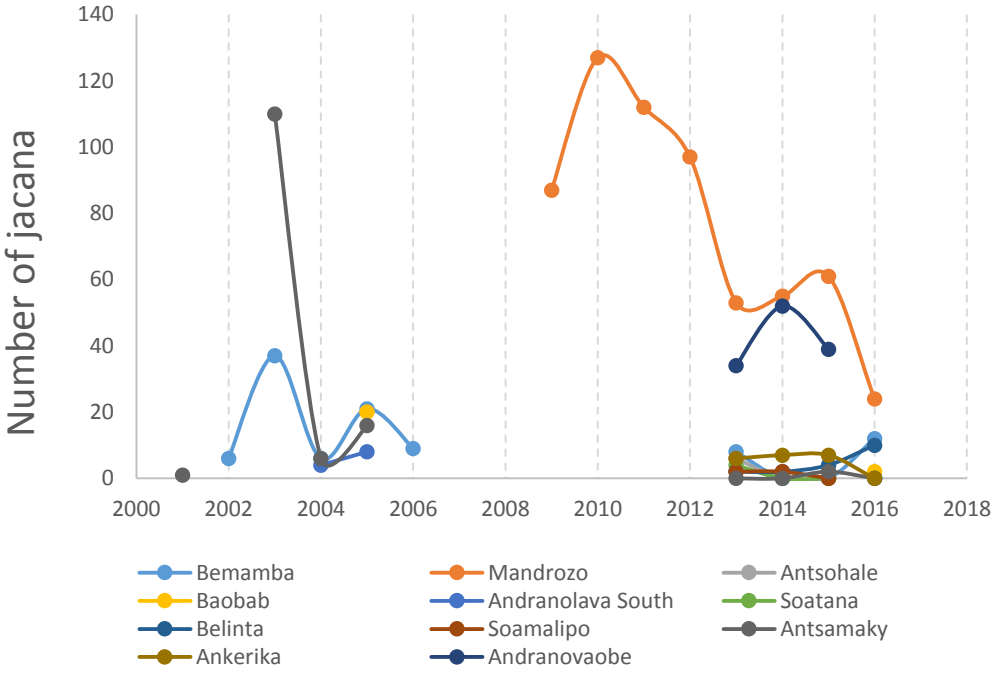
Lake	Longitude	Latitude
Lake Antsamaky	44.36	-19.04
Lake Ankerika	44.45	-19.02
Lake Bemamba	44.36	-18.84
Lake Belinta	44.43	-19.05
Lake Andranovorilava	49.73	-12.8
Soatana	44.4857	-19.069
Mandrozo	44.0749	-17.559
Tsimembo Manambolomaty	44.5719	-19.117
Soamalipo	44.4052	-19.045
Mandrozo	44.0638	-17.599
Andranovorindremalaza allee du baobab	44.4379	-20.229

Table S5.2. Lakes visited during January – October 2016 to survey the Madagascar jacana (*Actophilornis albinucha*) and their presence. Long= degrees longitude, lat = degrees latitude**.**

Site	Long	Lat	Present= 1 Absent = 0	Total N° individuals
Lake Anjanjany	49.54	-12.55	0	NA
Lake Ambia	49.51	-12.54	0	NA
Lake Matsaboribe	49.48	-12.54	0	NA
Lake Papan'i JAO	49.53	-12.53	0	NA
Lake Maivadoany	49.90	-13.15	0	NA
Lake Andohanampagnasy	49.90	-13.12	0	NA
Lake Andohazavy	49.91	-13.12	0	NA
Lake Mitohitohy	49.74	-12.82	0	NA
Lake Andranovorilava	49.73	-12.80	0	NA
Lake around Ambanja	48.45	-13.67	0	NA
Lakes beside the National road between Port-Berger and Antsohihy	47.88	-15.33	0	NA
Lake Tsinjomitondraka North	47.12	-15.66	0	NA
Lake Andranolava	47.11	-15.64	0	NA
Lake Kinkony	45.91	-16.15	0	NA
Lake Amboromalandy	46.76	-16.13	0	NA
Lakes beside the National road between Port-Berger and Mampikony	47.62	-15.57	0	NA
Lakes beside the National road between Matsaborilava and Port-Berger	47.58	-15.77	0	NA
Lakes beside the National road from Mampikony to Amboromalandy	47.65	-16.83	0	NA

Site	Long	Lat	Present= 1 Absent = 0	Total N° individuals
Lake Andranolava	44.41	-19.10	0	NA
Lake Antsamaky	44.36	-19.04	0	NA
Lake Ankerika	44.45	-19.02	0	NA
Lake Ankilizato north	43.25	-22.11	0	NA
Lake Ambondro	45.02	-20.42	0	NA
Lake Croisement Beiky	45.04	-20.41	0	NA
Lake Angodogodo	45.04	-20.41	0	NA
Lake Mavogisa	45.02	-20.41	0	NA
Lake Ambaibolava	45.63	-20.34	0	NA
Lake Andriamondra	45.55	-20.15	0	NA
Ponds around Allee du Baobab	44.43	-20.22	0	NA
Lake Sirave	43.90	-20.90	0	NA
Lake Ambondro	43.90	-20.89	0	NA
Lake Andasakoa	43.64	-22.02	0	NA
Lake Andramagnobe	43.59	-21.89	0	NA
Lake Andramagnokely	43.59	-21.89	0	NA
Lake Andramagnokely	43.59	-21.89	0	NA
Lake Andramagnokely	43.59	-21.89	0	NA
Lake Anosy	49.90	-13.14	1	2
Lake Ambinagny	49.96	-13.13	1	1
Lake Matsaborilava	47.56	-15.76	1	3
Lake Tsinjomitondraka South	47.12	-15.67	1	13
Lake Madiromilomboka	46.77	-16.15	1	4
Lake Ampisaraha	46.76	-16.14	1	2
Lake Marogoaky	46.77	-16.14	1	8
Lake Bejio Est	44.12	-17.57	1	7
Lake Bejio Ouest	44.11	-17.57	1	3
Lake Ampiliravao	44.05	-17.55	1	1
Lake Mokotobe	44.06	-17.55	1	5
Lake Nosin'omby	44.07	-17.55	1	8
Lake Betakilotra	44.04	-17.54	1	10
Lake Bemamba	44.36	-18.84	1	12
Lake Belinta	44.43	-19.05	1	10
Lake Besitera	44.35	-19.04	1	4
Lake Ranovorindagory	45.54	-20.13	1	3
Lake Ambariratabe	44.79	-20.42	1	4
Lake Berano (Manamby)	44.79	-20.41	1	2
Lake Belalitra	45.62	-20.34	1	8
Lake Allée de Baobab	44.41	-20.26	1	2
Lake Andramagnokely	43.59	-21.89	1	23
TOTAL			**22**	**135**

(A)



(B)

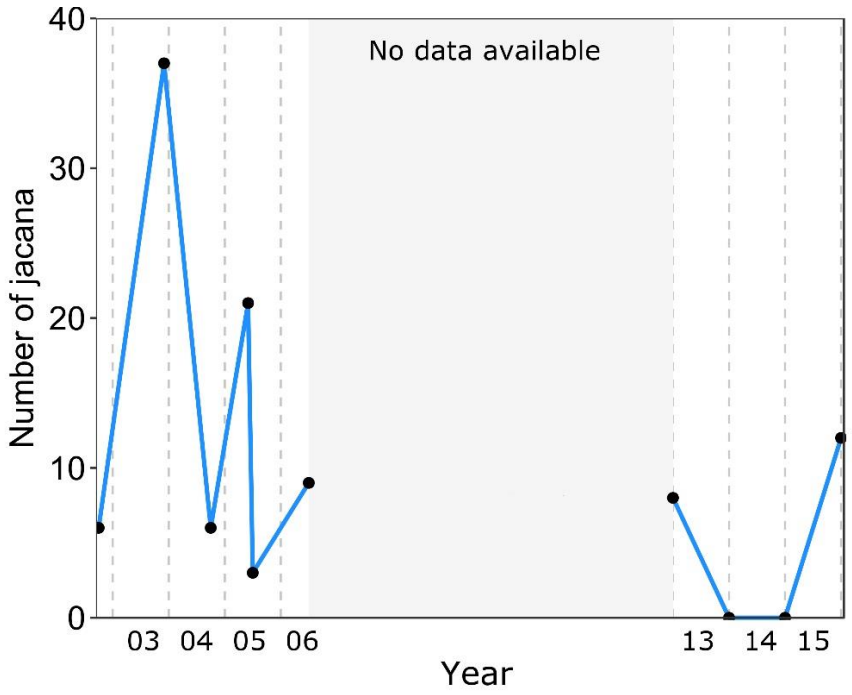


Figure S5.1. Madagascar jacana (*Actophilornis albincha*) presence records from repeated wetland bird surveys conducted by The Peregrine Fund and Durrell Wildlife Conservation Trust. A) Data from 11 lakes (Table S5.1) between 2001 and 2016 and B) Lake Bemamba only, as this lake had the most consistent data available. Dotted lines indicate year boundaries, data started in 2002 and ends in 2016. Grey area between July 2006 and 2013 is when no data for Madagascar jacana were available.

Figure S5.2 Immature Madagascar jacana (*Actophilornis albinucha*) plumage variation. (A) shows immature plumage and (B) shows intermediate plumage between immature and adult.

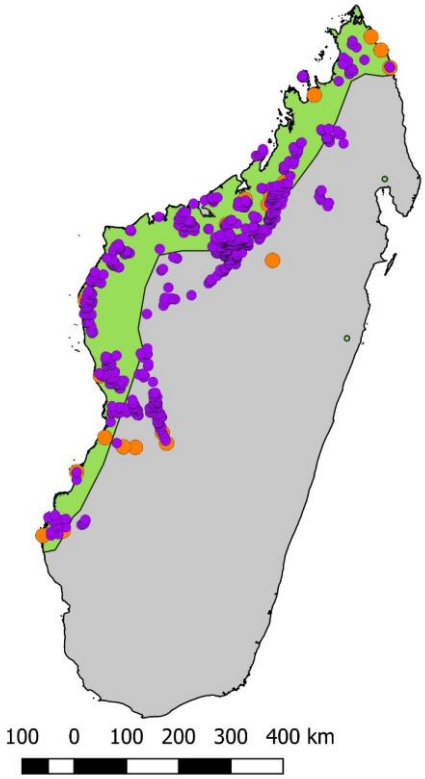


Figure S5.3. Map of Madagascar wetland locations which were included to estimate population size of the Madagascar jacana (*Actophilornis albinucha*) (purple dots). Orange dots are the location of sites surveyed during 2016 for Madagascar jacana. Green shading indicates the predicted range of Madagascar jacana (BirdLife International 2016).

Post Chapter 5 commentary

This chapter focused on understanding the basic ecology of the Madagascar jacana. In the previous chapter (Chapter 4), I assumed the Madagascar jacana shared mating system of classical polyandry with the African jacana. Using combined genetic (Chapter 4), phylogenetic, and morphometric (this Chapter) data I am confident that the Madagascar jacana also exhibits sex-role reversal and classical polyandry. However, field studies of breeding behaviour should be conducted to test the reliability of these approximate measures of mating system. In addition, observing jacana outside of the current expected range could support the finding of a population expansion indicated in Chapter 4.

6

Discussion

What are the population genetic consequences of mating systems and dispersal?

Mating systems, dispersal and gene-flow are inherently linked. In this thesis I aimed to demonstrate the benefits of incorporating macro- and micro-evolutionary data in addition to intra-genomic data in a holistic approach to understand the genetic consequences of mating systems. This thesis provides novel evidence of how mating systems have profound consequences at every level of genetic diversity, from within-genome (Chapters 3 and 4), to within species (Chapters 2-4), to across species (Chapters 2 and 4). In addition, genetic and demographic results (Chapters 3 and 5) have important implications for conservation management.

My main findings were:

- Chapter 2: polygamy promotes genetic homogenisation in plovers and monogamy is associated with stronger population differentiation in shorebirds. Migratory behaviour, however, has no association with diversification in shorebirds.
- Chapter 3: snowy plover subspecies are genetically supported and genetic differentiation is correlated with geographic distance. Recent population bottlenecks were found in all subspecies but were most severe in *Charadrius nivosus tenuirostris*. The high Z chromosome diversity found, reflects the polyandrous mating system of this species.
- Chapter 4: intense simultaneous polyandry has a strong elevating effect on relative Z chromosome to autosome diversity in *Actophilornis* jacanas, however, demographic expansions could also play an important role in increasing Z chromosome diversity. Intra- and inter-specific divergence is lower on the Z chromosome compared to the autosome. Southern African jacana populations and the Madagascar jacana are both characterised by high gene-flow. Genetic diversity is lower in the Madagascar jacana compared to the African jacana.
- Chapter 5: The Madagascar jacana exhibits strong sexual size dimorphism where females are significantly larger than males. This species also conforms to the "island rule" of being larger than its closest mainland relative. Population size is estimated between ca. 1,000 - 2,000 individuals and my work suggests there is an urgent need for a dedicated reassessment of Madagascar jacana threat status.

Mating systems and diversification

Sexual selection has been associated with driving speciation since its conception, yet the evidence for this form of selection alone leading to novel species is often questioned (e.g. Morrow *et al.* 2003, van Doorn *et al.* 2004, Servedio 2011, Servedio and Kopp 2012, Servedio and Boughman 2017). In this thesis I suggest two ways in which sexual selection can limit diversification. Firstly, in Chapter 2, I present, and find support for, the "dispersal to mate" hypothesis which explains how sexual selection may increase gene-flow between breeding populations, counteracting any accumulated differences. Secondly, I show that at the intra-genomic level, polyandry can reduce population divergence on the Z chromosome (Chapter 5), whereas, polygyny and monogamy are associated with its increase (e.g. Mank *et al.* 2007, Meisel and Connallon 2013, Wright *et al.* 2015, Irwin 2018, Van Belleghem *et al.* 2018). Here, I will discuss these, in additional to other findings, and suggest alternative hypotheses.

Breeding dispersal, and any resulting gene-flow, are powerful evolutionary and demographic forces that shape population differentiation and ultimately, biodiversity (Clobert *et al.* 2012). Associating mating systems with population differentiation has, however, been neglected in the study of sexual selection and speciation (Maan and Seehausen 2011). To explain how breeding dispersal can reduce diversification, in Chapter 2 I tested the hypothesis that that due to the high pressure for multiple breeding partners in polygamous species, individuals disperse to multiple breeding sites to increase reproductive success. This leads to higher gene-flow between populations of polygamous species (decreasing or limiting divergence) compared to equivalent breeding populations of monogamous species (Figure 6.1, Chapter 2). Although speciation with gene-flow is possible (reviewed by Rice and Hostert 1993, Feder *et al.* 2012), selection or divergence on co-varying sexual traits and preferences must be strong and/or reflect local adaptation in order to maintain or create independent species complexes (Servedio and Boughman 2017).

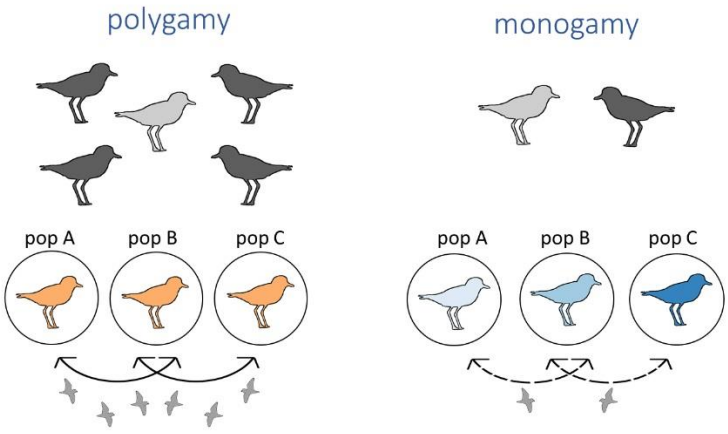


Figure 6.1. Schematic diagram of the "dispersal-to-mate" hypotheses. This hypothesis predicts polygamous species will be characterised by high gene-flow resulting in genetic homogeneity compared to low gene-flow and high structure in monogamous species.

In the subsequent Chapters 3 and 4, I found mixed support for this hypothesis in polyandrous species based on autosomal markers. Madagascar jacana was characterised by genetic homogeneity (Chapter 4), whereas, genetic structure was present in the snowy plover and African jacana (Chapter 3 and 4). Extending the findings of Chapter 2 to other taxa, I find mixed support. For example, the highly polygynous Ruff (*Philomachus pugnax*), is characterised by high gene-flow and no genetic structure (Verkuil *et al.* 2012), and a similar lack of structure is found in polygynous White-throated capuchins (*Cebus capucinus*) (Ruiz-García and Castillo 2016). However, movement data from the polygynous pectoral sandpiper showed males travel widely during a single breeding season to visit multiple populations (Kempenaers and Valcu 2017). In contrast, one of the most well-known examples of extreme monogamy and strong site fidelity, the Emperor Penguin (*Aptenodytes forsteri*), has very weak genetic structure among breeding colonies (Younger *et al.* 2017), as do strictly monogamous Black-footed Albatross (*Phoebastria nigripes*, Dierickx *et al.* 2015).

Although on a case-by-case basis genetic structure may or may not be present, it is the relative degree of population differentiation between monogamous and polygamous species that should be examined to test this hypothesis. Directly comparing ecologically distinct species is likely to be confounded by other ecological, anthropogenic, and environmental factors shaping differentiation (discussed below). To test the "dispersal-to-mate" hypothesis, here I reduced the confounding factors by comparing genetic data from a behaviourally diverse, yet ecologically similar group of species (Chapter 2). Such directly comparable groups of species with available genetic data are rare, and this itself may restrict mating system comparisons between other taxa. The association between mating system and gene-flow that I describe here, may also be restricted to taxa with high dispersal capabilities and/or long breeding seasons, which enable individuals to take advantage of optimal reproductive conditions to maximise their fitness.

Gene-flow can be a powerful population genetic process and just a few dispersal events are required to cause large changes in allelic frequencies (Mills and Allendorf 1996). These movements are continuous throughout an animal's lifetime and involve not just individual decisions for breeding, but also species and environmental level interactions (Clobert *et al.*, 2012). For example, movement can be driven by physical capability (e.g. Moore *et al.* 2008, Claramunt *et al.* 2012, Weeks and Claramunt 2014), niche adaptation (e.g. Salisbury *et al.*, 2012), personality (e.g. Cote *et al.* 2010, Denoël *et al.* 2018), seasonal habitat availability (e.g. Pedler *et al.* 2014), and the presence or absence of biogeographic features (e.g. Munshi-South *et al.* 2016). Furthermore, inbreeding avoidance has been documented as a major driver of natal dispersal which is the movement of individuals away from their birth site (Packer 1984, Pusey 1987, Dobson 2013, Moussy *et al.* 2013, Blyton *et al.* 2015). It is not surprising, therefore, that highly vagile species lack population differentiation over long distances (Belliure *et al.* 2000, Claramunt *et al.* 2012, Medina *et al.* 2018), especially when their habitats are naturally ephemeral (Chapter 4, Tarboton 1995). Alternatively, the pattern of isolation by distance (Wright 1943), is common across animal taxa (Orsini *et al.* 2013, Aguillon *et al.* 2017) and I found evidence of this regardless of mating system (Chapters 2- 4). The age of dispersal within a lifetime may also affect the influence of dispersal on gene-flow patterns. For example, if natal dispersal is more costly to survival compared to adult breeding dispersal, the relative

influence of breeding dispersal on gene-flow would be stronger than that of natal dispersal. However, to compare the relative contribution of natal and breeding dispersal to population genetic consequences, one would have to account for dispersal distance, which is often longer for natal dispersal (Paradis *et al.* 1998). Disentangling the drivers and relative contribution of natal or breeding dispersal to gene-flow patterns requires comprehensive long term mark-recapture studies combined with genetic pedigrees. Due to the challenges of detailed long term ecological studies (reviewed by Kuebbing *et al.* 2018), such detailed investigations are usually restricted to single populations (e.g. Garant *et al.* 2005), which limits our understanding of how dispersal can influence multi-population level genetic structure.

The spectrum of genetic differentiation is the combined outcome of factors including life history traits, demography and environment (Orsini *et al.* 2013, Carroll *et al.* 2015, Harvey and Brumfield 2015). Although it is clear from this study and others (e.g. Parreira and Chikhi 2015, Shaw *et al.* 2018) that mating systems can have a strong influence on population structure, breeding dispersal may play a relatively minor role in shaping the population genetic structure of a species compared to other factors. As an alternative to the "dispersal-to-mate" hypothesis, if the acquisition of territories (e.g. leks) increases access to the opposite sex in highly polygamous species, dispersal may instead be restricted to ensure high quality territory ownership (Harts *et al.* 2015).

In addition to restrictors or barriers to movement, our interpretation of population genetic patterns is often dictated by the informative power of the genetic markers (Sunnucks 2000). From Chapter 2 through to Chapter 4, I substantially increased the number of genetic markers analysed and support well-established evidence that with more markers, comes greater power to detect fine scale structure (Chapter 3, Ruegg *et al.* 2014, Saenz-Agudelo *et al.* 2015, Jahner *et al.* 2016, Barth *et al.* 2017, Vendrami *et al.* 2017, Younger *et al.* 2017). When using high throughput genomic methods (Chapters 3 and 4), both the number and the breadth of genetic markers is increased across the genome, compared to traditional methods e.g. microsatellites (Andrews *et al.* 2016). This includes sequencing loci on the sex chromosomes (e.g. Evans *et al.* 2014), which allows the examination of sex-biased dispersal hypotheses (e.g. Lavretsky *et al.* 2015). One important assumption of the "dispersal to mate" hypothesis presented in Chapter 2, is that gene-flow is driven by the polygamous sex, to increase mating success. To provide estimations of female biased, male biased, and non sex-biased gene-flow, I compared genetic structure and isolation by distance between mtDNA, Z-linked loci, and autosomal loci respectively (Prugnolle and Meeûs 2002, Aguillon *et al.* 2017). I found inconclusive support for female biased gene-flow in the three polyandrous species tested (Chapters 3 and 4). In snowy plovers, the assignment of individuals to their respective subspecies was improved with Z-linked markers compared to mtDNA, and within *C. n. nivosus,* Z-linked markers provided a strongest isolation by distance pattern. Together these support a role of female biased dispersal in snowy plovers, however, this result may also be an artefact of a low number of loci (n=65) and individuals (n=25) in the Z-linked analyses.

Using a similar comparative marker approach in *Actophilornis* jacanas, I found no evidence of sex-biased dispersal in the Madagascar jacana, which was found to be

genetically homogenous with all markers (Chapter 4). In their mainland sister species, the African jacana, isolation by distance was stronger with autosomal loci compared to Z-linked loci, suggesting male biased dispersal. Alternatively, the lack of mtDNA spatial structuring in southern populations, could suggest female biased dispersal. These conflicting patterns suggest gene-flow is high in both sexes of *Actophilornis* jacanas.

Taken together, my studies were limited in their power to assess if polyandry is associated with female biased dispersal, however, detecting female biased dispersal in ZW systems is challenging for three main reasons. Firstly, the small size of the female specific W chromosome relative to the genome (Rutkowska *et al.* 2012) means that low depth RADseq methods may not produce reliable sequencing coverage for this region (but see Baxter *et al.* 2011). Secondly, the presence of multiple Z chromosome non-recombining paralogues on the W chromosome (Smeds *et al.* 2015) makes identifying W specific loci using alignment methods difficult without a reliable W chromosome reference sequence. Thirdly, recent analysis strongly disputes the reliability of mtDNA to detect isolation by distance (Teske *et al.* 2018). Furthermore, female-biased dispersal is common among avian families (Greenwood 1980, Greenwood and Harvey 1982, Clarke *et al.* 1997, but see Mabry *et al.* 2013), therefore, even if detected, disentangling the drivers of this behaviour is challenging.

The importance of population level analyses

The role of behaviour in speciation is increasingly documented (Uy *et al.* 2018) and meta-analyses testing the relationship between sexual selection and speciation are common (e.g. Morrow *et al.* 2003, Ritchie 2007, Maan and Seehausen 2011, Kraaijeveld *et al.* 2011, Jennions *et al.* 2012, Rodríguez *et al.* 2013, Huang and Rabosky 2014, Ellis and Oakley 2016, Janicke *et al.* 2018). However, due to the plethora of complex species specific traits and unique evolutionary history, using higher taxonomic levels limits our ability to determine the mechanisms behind diversification. Alternatively, as a precursor to speciation (Harvey *et al.* 2017), exploring the potential drivers of genetic population differentiation, can improve our understanding of the role sexual selection has in shaping biodiversity.

Much of the restriction on population level analysis of sexual selection is due to the availability of samples. For example, only one breeding population may be required to predict a mating system, and only one sample can represent an entire species in a species based phylogenetic approach. Multi-population level assessments are, therefore, more difficult to gather data for. Furthermore, the reliability of conclusions drawn from population genetic studies are dependent on the distribution of sampling sites (Younger *et al.* 2017, Bradburd *et al.* 2018). For example, in Chapters 2-4 the sampling distribution of some species (e.g. African jacana, snowy plover, chestnut banded plover) limit how accurate we can be about inferring the population genetic structure and gene-flow (Beerli 2004, Slatkin 2005). Particularly in Chapter 4, the area of the missing sampling sites for RADseq covers > 5,500 km. Therefore, rather than there being a strong barrier to dispersal present, African jacanas may be characterised by a gradual change in allelic frequencies. This methodological issue is highly important for the delineation of conservation units (Waples and Gaggiotti 2006, Bradburd *et al.* 2018). In Chapter 3 for example, we recommended Florida snowy plovers as a separate management unit based

on the genetic differentiation between them compared to the rest of the *C. n. nivosus* sampling sites. However, the remaining *C. n. nivosus* sampling sites do not fully represent the inland USA populations. To be justified in delineating legislative protection to conservation units, a stratified sampling approach is required. This is often an idealistic requirement which is not possible to implement in studies due to restraints on sampling collection and the financial cost of field work. In these cases, museums and biobanks provide a vital resource for additional samples (Holmes *et al.* 2016).

Intra-genomic diversity and divergence

I present here findings that within polygamous mating systems, the direction of sexual selection pressure (i.e. on males or females) can result in alternative accelerating or decelerating sequence evolution in the Z chromosome (Chapter 5, Wright *et al.* 2015). Examining the effect of classical polyandry in the divergence of *Actophilornis* jacanas revealed that the intra- and inter- specific genetic distance of Z chromosome loci was lower than that of autosomal loci (Chapter 5). Reduced divergence on the Z chromosome contrasts with the growing body of research from monogamous and polygynous species describing the Z chromosome as having a proportionally greater role in speciation, as a result of "Fast-Z" evolution and male mutation bias (reviewed by Irwin 2018). In contrast to classically polyandrous systems, in polygynous mating systems, female biased sex ratio (Liker *et al.* 2013) and high variance in male mating success reduces the effective population size of the Z chromosome (Caballero 1995, Charlesworth 2001, Ellegren 2009, Corl and Ellegren 2012). Consequently, the effect of purifying selection is weaker on this chromosome due to lower diversity (Caballero 1995, Laporte and Charlesworth 2002), therefore, the power of genetic drift is stronger (Vicoso and Charlesworth 2009), resulting in faster Z chromosome divergence (Mank *et al.* 2007, Wright *et al.* 2015). However, Z chromosome diversity is increased in polyandrous species (Chapter 3 and 4) which I predict enhances purifying selection and weakens genetic drift, therefore, reducing divergence rates (Vicoso and Charlesworth 2009). Reduced divergence on the Z chromosome adds further complexity to the role sexual selection plays in diversification, and indicates that at the same genomic scale, but in polygynous rather than polyandrous systems, sexual selection can act contrastingly to promote or reduce differentiation. It is, however, important to note that although role of the sex chromosome in population divergence can be disproportionately higher than the autosomes, sex chromosomes are relatively small parts of the nuclear genome and are, therefore, unlikely to drive macro-evolution alone (Irwin 2018). Furthermore, in the snowy plover the Z chromosome F_{ST} between populations was higher than that of the autosomes (Chapter 3), although, this could be an artefact of the large difference in the number of markers between the datasets (Z chromosome: 65, autosome: 789).

To my knowledge, I present here the first empirical evidence that classical polyandry elevates the relative Z chromosome to autosome diversity in natural populations. Elevated Z chromosome diversity was confirmed in three classically polyandrous species representing both sequential (Chapter 3) and simultaneous (Chapter 4) breeders. I hypothesise that this increase is caused by a heavily male biased adult sex ratio, in addition to high variance in female relative to male breeding success (Figure 6.2). This novel result complements the growing body of empirical and theoretical evidence which demonstrate the far reaching effects of sexual selection, from the

interspecific level, to intra-genomic variation. Furthermore, it demonstrates the importance of studying a full representation of breeding systems when investigating the genomic consequences of sexual selection. Both the snowy plover and the African jacana have similarly biased adult sex ratios (proportion of males in the population) of 0.63 (Eberhart-Phillips *et al.* 2017) and 0.65 (Tarboton 1992) respectively. However, by exhibiting simultaneous, rather than sequential polyandry, female sexual selection pressure is extremely high in jacanas (Kvarnemo and Simmons 2013). The intensity of sexual selection pressure in snowy plovers and jacanas is reflected in the degree of departure from a neutral N_Z/N_A diversity ratio of 0.75 under equal male and female mating opportunities. In the snowy plover Watterson's theta N_Z/N_A ratio ranged from 0.75 to 1.10 (Chapter 3), whereas, this was 1.30 in the African jacana and rose to a staggering 1.67 in the Madagascar jacana (Chapter 4). Although population expansions may have had some role in elevating the relative N_Z/N_A ratio in *Actophilornis* jacanas, the evidence of population bottlenecks in snowy plover populations, indicates that demographic growth cannot explain the high Z chromosome diversity alone. No study that I am aware of has found N_Z/N_A ratio as high as I describe here and I strongly encourage a full assessment of N_Z/N_A ratio across the whole spectrum of mating systems to put these values into perspective. However, due to the lack of an annotated reference genome for jacanas or snowy plover, it is possible that the methods employed here were not stringent enough to isolate only Z chromosome loci. For example the putative Z chromosome loci may include areas of the pseudo-autosomal region which would increase the N_Z/N_A diversity ratio. The genomic architecture of the pseudo-autosomal region which is recombining and present in both sex chromosomes is little is understood (reviewed by Otto *et al.* 2011), and can require significant genetic resources to identify (Smeds *et al.* 2014) which were not available for this investigation.

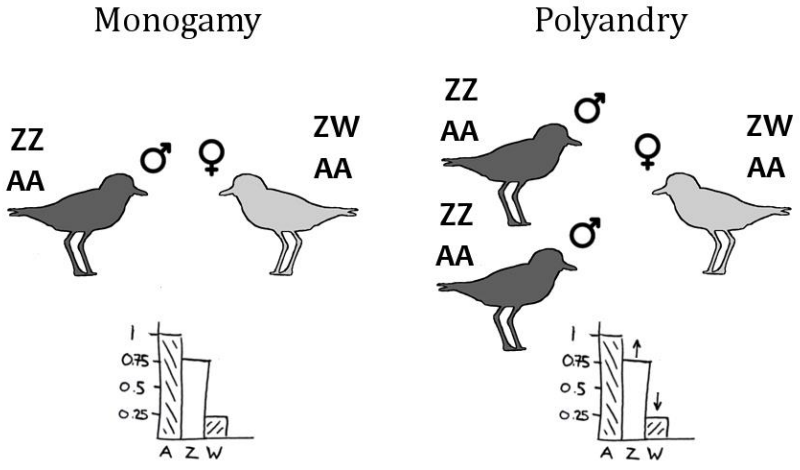


Figure 6.2. Schematic diagram illustrating the influence male biased adult sex ratio can have on the relative genetic diversity of the autosomes (A) and sex chromosomes (Z= Z chromosome, W= W chromosome) in female heterogametic systems.

The association between polyandry and elevated N_Z/N_A ratio is exclusive to ZW systems, as male biased sex ratios in XY systems would reduce effective population size of the X chromosome. In terms of relative X chromosome to autosome diversity, in the absence of male mutation bias, ZW polyandry is equivalent to XY polygyny (Ellegren 2009, Corl and Ellegren 2012). Much of the research into relative diversity of sex chromosomes and autosomes in XY systems has focused on the effect of demography and selection rather than mating systems (e.g. Gottipati *et al.* 2011, Arbiza *et al.* 2014, Chen *et al.* 2018, but see Evans *et al.* 2014). However, variance in male reproductive success and female biased sex ratios are proposed to explain elevated X chromosome to autosome diversity in human societies (Hammer *et al.* 2008), *Drosophila* (Singh *et al.* 2007) and *Teleogryllus* crickets, even when accounting for population expansions (Moran *et al.* 2018).

Assumptions

Throughout this thesis I have used genetic data to understand the consequences of dispersal in relation to mating systems by making two major assumptions. Firstly, like many population genetic studies, I predicted the degree of dispersal using gene-flow as a proxy (Slatkin 1985). Although genetic data can give us an insight into the evolutionary outcomes of movement, the accuracy of this proxy genetic measure alone cannot provide robust estimates of actual population connectivity (reviewed by Lowe and Allendorf 2010). To do this we need to combine gene-flow data with movement information (e.g. mark recaptures and remote tracking), and demographic parameters such as the relative contributions of local, versus migrant, reproductive outputs (Hughes 1998, Lowe and Allendorf 2010, Habel *et al.* 2015, Jønsson *et al*. 2016, Cayuela *et al.* 2018).

Secondly, I assume that the polygamous sex will gain a greater fitness reward by visiting multiple populations compared to breeding in the same population, as dispersing outside of familiar territories in search of mating opportunities can be costly (Young and Monfort 2009). However, there is little empirical evidence of increased reproductive success after breeding dispersal in polygamous species (but see Valcu and Kempenaers 2008), and dispersal between breeding attempts in the same season is rarely documented (e.g. Székely and Lessells 1993, Howlett and Stutchbury 1997, Pearson and Colwell 2014, Williams and Boyle 2018).

Regardless of mating system, breeding dispersal, between and within seasons, is more commonly associated with females compared to males, and has been related to previous nest failure or low success, (Part and Gustafsson 1989, Robinson and Oring 1997, Forero *et al.* 1999, Pearson and Colwell 2014, Terraube *et al.* 2015, Becker *et al.* 2018, but see Howlett and Stuchbury 1997, Flodin *et al.* 2012). Furthermore, breeding dispersal does not necessarily predict if a reproductive attempt is made, nor if it was successful (Robertson *et al.* 2018). Testing the validity of the relationship between mate change, dispersal and breeding success will require comparative longitudinal data encompassing multiple breeding habitats across the range. However, the financial and logistical constraints of long term dispersal studies limit our ability to thoroughly assess these relationships. To tease apart the drivers and consequences of breeding dispersal, and validate both of these assumptions, we require a combination of field studies, direct tracking, and measures of gene-flow.

This thesis highlights a clear deficiency in our basic knowledge of shorebird biology, particularly in respect to mating systems (Chapters 2 and 5). Accurate estimates of mating systems were found for less than half of extant shorebird species (Chapter 2), and like many tropical species, the Madagascar jacana was hardly understood prior to my investigations (Chapters 4 and 5). Elevated Z chromosome diversity (Chapter 4) and reversed sexual size dimorphism (Chapter 5) in the Madagascar jacana provide compelling evidence of extreme polyandry in this species. However, field investigations of the breeding biology of the Madagascar jacana and many other taxa are required to improve our understanding of the evolution and consequences of mating systems. Natural history studies have reduced in recent years across taxa in favour for more "sexy science" such as molecular ecology (McMallum and McCallum 2006, e.g. Chapters 3 and 4). Although there is no doubt in the advantages of novel methods (e.g. Wilkinson *et al.* 2015, Allendorf 2016), field studies provide invaluable and fundamental data to develop hypotheses and interpret biological discoveries (Hughes 1998, Delia *et al.* 2017, Kuebbing *et al.* 2018). More importantly, without census estimations of population size, trends, and threats, we are unable to efficiently implement or prioritise conservation measures (IUCN 2001, Andres *et al.* 2012, Chapter 5).

Implications for conservation

The importance of understanding evolutionary processes, lies in the application of this knowledge to conserve biodiversity. Here, in addition to contributing to pure evolutionary research I have demonstrated ways in which this information can be utilised for conservation management. Effective dispersal, whereby dispersal leads to reproductive success, is essential to retain population connectivity and persistence (Lowe and Allendorf 2010). If dispersal propensity is predicted by certain traits, such as mating systems, this information could be used identify priority species of conservation concern (i.e. those with isolated populations which are susceptible to inbreeding). My findings suggest that polygamous species may be less likely to suffer from the genetic effects of isolation and inbreeding compared to monogamous species, due to high gene-flow (Chapter 2). However, this is not always the case. For example, in the polyandrous snowy plover, measuring genetic differentiation indicated that rather than species wide genetic homogeneity, fine scale structure exists, and demographic analysis strongly suggests recent and widespread population bottlenecks have occurred across their range (Chapter 3). Although both jacana species show genetic signals of expansion (Chapter 4), the lack of any mtDNA diversity in the Madagascar jacana (Chapter 4), its small census population size (Chapter 5) and the pervasive threats to Madagascar wetlands (Bamford *et al.* 2017) suggest the threat status of this species should be thoroughly examined.

Future studies

Throughout Chapters 2-5 I have highlighted several avenues of future research, to summarise I propose the following as the most important areas to continue this research:

- Combine field studies of breeding behaviour with gene-flow and direct movement data to integrate individual, sex specific breeding decisions with dispersal and the population genetic consequences.

- Expand tests of the "dispersal to mate" hypothesis across more comparable groups of taxa.
- Compare the relative Z chromosome and autosomal diversity between monogamous relatives of the snowy plover and *Actophilornis* jacanas (e.g. the Lesser jacana, *Microparra capensis* and white fronted plover, *Charadrius marginatus*).

The findings from my thesis provide novel hypotheses by which social behaviour can be a powerful predictor of genetic diversity and differentiation patterns. In wild populations, however, the forces isolating or homogenising populations are extremely complex and can show different signals depending on the scale of analyses. This thesis demonstrates that although there is no "one size fits all" answer to understanding the drivers of population differentiation and genetic diversity, the ultimate goal of evolutionary research should be how we can apply this information to conserve it (Smith *et al.* 1993, Winter *et al.* 2012, Pierson *et al.* 2015, Haig *et al.* 2016, Hunter *et al.* 2018).

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Appendix I

Hudson, M.A., Young, R.P., **D'Urban Jackson, J**., Orozco-terWengel, P., Martin, L., James, A., Sulton, M., Garcia, G., Griffiths, R.A., Thomas, R., Magin, C., Bruford, M.W., and Cunningham A.A. 2016. Dynamics and genetics of a disease-driven species decline to near extinction: lessons for conservation. *Scientific Reports*. 6: 30772 doi:10.1038/srep30772

Abstract

Amphibian chytridiomycosis has caused precipitous declines in hundreds of species worldwide. By tracking mountain chicken (*Leptodactylus fallax*) populations before, during and after the emergence of chytridiomycosis, we quantified the real-time species level impacts of this disease. We report a range- wide species decline amongst the fastest ever recorded, with a loss of over 85% of the population in fewer than 18 months on Dominica and near extinction on Montserrat. Genetic diversity declined in the wild, but emergency measures to establish a captive assurance population captured a representative sample of genetic diversity from Montserrat. If the Convention on Biological Diversity's targets are to be met, it is important to evaluate the reasons why they appear consistently unattainable. The emergence of chytridiomycosis in the mountain chicken was predictable, but the decline could not be prevented. There is an urgent need to build mitigation capacity where amphibians are at risk from chytridiomycosis

Appendix II

Hou, X., Xu, P., Lin, Z., **D'Urban Jackson, J**., Dixon, A., Bold, B., Xu, J. and Zhan, X. (2018). An integrated tool for microsatellite isolation and validation from the reference genome and their application in the study of breeding turnover in an endangered avian population. *Integrative Zoology* doi: 10.1111/1749-4877.12305.

Abstract

Accurate individual identification is required to estimate survival rates in avian populations. For endangered species, non-invasive methods of obtaining individual identification, such as using molted feathers as a source of DNA for microsatellite markers, are preferred because of less disturbance, easy sample preparation and high efficiency. With the availability of many avian genomes, a few pipelines isolating genome-wide microsatellites have been published, but it is still a challenge to isolate microsatellites from the reference genome efficiently. Here, we have developed an integrated tool comprising a bioinformatic pipeline and experimental procedures for microsatellite isolation and validation based on the reference genome. We have identified over 95,000 microsatellite loci and established a system comprising ten highly polymorphic markers (PIC value: 0.49-0.93, mean: 0.79) for an endangered species, saker falcon (*Falco cherrug*). These markers (except one) were successfully amplified in 126 molted feathers, exhibiting high amplification success rates (83.9-99.7%), high quality index (0.90-0.97) and low allelic dropout rates (1-9.5%). To further assess efficiency of this marker system in a population study, we identified individual sakers using these molted feathers (adult) and 146 plucked feathers (offspring). The use of parent and offspring samples enabled us to infer the genotype of missing samples (N = 28), and all adult genotypes were used to ascertain that breeding turnover is a useful proxy for survival estimation in sakers. Our study presents a cost-effective tool for microsatellite isolation based on publicly available reference genomes and demonstrates the power of this tool in estimating key parameters of avian population dynamics.